Supporting Information for

"Protein farnesyltransferase catalyzes unanticipated farnesylation and geranylgeranylation of shortened target sequences"

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Table S1. Yeast plasmids used in this study.

Plasmid	**Genotype**	**Reference**
p4339	*NAT*^{R}	[1]
pRS315	*CEN LEU2 (vector)*	[2]
pRS316	*CEN URA3 (vector)*	[2]
pRS426	*2μ URA (vector)*	[2]
pSM217	*2μ URA3 (vector)*	[3]
pSM1194	*2μ URA3 STE24*	[4]
pWS114	*2μ URA3 RCE1*	This study
pWS610	*CEN LEU2 MFA1*	[5]
pWS612	*CEN LEU2 MFA1-CASQ*	[5]
pWS942	*CEN URA3 YDJ1*	[6]
pWS1024	*CEN LEU2 MFA1-C(TAG)IA*	[7]
pWS1025	*CEN LEU2 MFA1-CV(TAG)*	This study
pWS1026	*CEN LEU2 MFA1-CVI(TAG)*	This study
pWS1079	*CEN LEU2 MFA1-CVI*	This study; **a**-factor screen
pWS1081	*CEN LEU2 MFA1-CCI*	This study; **a**-factor screen
pWS1082	*CEN LEU2 MFA1-CII*	This study; **a**-factor screen
pWS1084	*CEN LEU2 MFA1-CRI*	This study; **a**-factor screen
pWS1085	*CEN LEU2 MFA1-CLA*	This study; **a**-factor screen
pWS1086	*CEN LEU2 MFA1-CLV*	This study; **a**-factor screen
pWS1087	*CEN LEU2 MFA1-CFY*	This study; **a**-factor screen
pWS1088	*CEN LEU2 MFA1-CLI*	This study; **a**-factor screen
pWS1132	*CEN URA3 YDJ1-SASQ*	[6]
pWS1138	*CEN LEU2 MFA1-CTI*	This study; **a**-factor screen
pWS1144	*CEN LEU2 MFA1-CFV*	This study; **a**-factor screen
pWS1171	*CEN URA3 YDJ1-CTI*	This study
pWS1172	*CEN URA3 YDJ1-CII*	This study
pWS1173	*CEN URA3 YDJ1-CFV*	This study

pWS1174	*CEN URA3 YDJ1-CVI*	This study
pWS1181	*CEN URA3 YDJ1-CNV*	This study; Ydj1p screen
pWS1209	*CEN URA3 YDJ1-CAL*	This study; Ydj1p screen
pWS1210	*CEN URA3 YDJ1-CLL*	This study; Ydj1p screen
pWS1211	*CEN URA3 YDJ1-CSV*	This study; Ydj1p screen
pWS1212	*CEN URA3 YDJ1-CQL*	This study; Ydj1p screen
pWS1213	*CEN URA3 YDJ1-CAF*	This study; Ydj1p screen
pWS1214	*CEN URA3 YDJ1-CVL*	This study; Ydj1p screen
pWS1216	*CEN URA3 YDJ1-CEV*	This study; Ydj1p screen
pWS1218	*CEN URA3 YDJ1-CFT*	This study; Ydj1p screen
pWS1221	*CEN URA3 YDJ1-CTV*	This study; Ydj1p screen
pWS1246	*CEN URA3 YDJ1-CTLM*	[6]
pWS1623	*CEN URA3 ydj1::NAT*^{R}	This study
pWS1767	*CEN URA3 RAM1*	This study
pWS1808	*2μ URA3 RAM1*	This study
pWS1811	*CEN LEU2 MFA1-AVI*	This study
pWS1812	*CEN LEU2 MFA1-SVI*	This study
pWS1813	*CEN URA3 YDJ1-AVI*	This study
pWS1814	*CEN URA3 YDJ1-SVI*	This study

Table S2. Yeast strains used in this study.

Strain	Genotype	Reference
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	[8]
IH1793	*MATα lys1*	[9]
RC757	*MATα sst2-1 rme his6 met1 can1 cyh2*	[10]
SM2331	*MAT***a** *trp1 leu2 ura3 his4 can1 mfa1 mfa2*	[3]
yWS304	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1::KAN*^{R}	[6]
yWS1632	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ram1::KAN*^{R}	[6]
yWS2542	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ram1::KAN*^{R} *ydj1::NAT*^{R}	This study
yWS2544	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1::NAT*^{R}	This study
yWS2938	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CTI*	This study
yWS2939	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CII*	This study
yWS2940	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CFV*	This study
yWS2941	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CVI*	This study
yWS2942	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CNV*	This study
yWS2943	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CAL*	This study
yWS2944	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CLL*	This study
yWS2945	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CSV*	This study
yWS2946	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CQL*	This study
yWS2947	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CAF*	This study
yWS2948	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CVL*	This study
yWS2949	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CEV*	This study

yWS2950	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CFT*	This study
yWS2951	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CTV*	This study
yWS2952	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-CRV*	This study
yWS2958	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-AVI*	This study
yWS2959	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1-SVI*	This study

Table S3. Impact of over-expressing prenylation pathway enzymes on CVI-dependent mating efficiency.[a]

Overexpression vector	**% mating (relative to empty vector)**
Empty vector	100 ± 23
RCE1	248 ± 31
STE24	73 ± 30
RAM1	103 ± 11

[a] Quantitative mating assays were performed using an **a**-factor CVI mutant as described in Materials and Methods. Values are the average of three biological replicates, with the error reflecting one standard deviation.

Table S4. Peptide information table for Cxx sequences tested in the human genome.

Human-derived sequences	Uniprot ID	Protein	Notes
CAF*	P36551	Oxygen-dependent coproporphyrinogen-III oxidase, mitochondrial	Isoform 2 only
	Q8NI17	Interleukin-31 receptor subunit alpha	Isoform 11 only
CAG	Q9NP78	ATP-binding cassette sub-family B member 9	Isoform 5 only
	A8MXD5	Glutaredoxin domain-containing cysteine-rich protein 1	Isoform 1 only
CAL*	Q9NUI1	Peroxisomal 2,4-dienoyl-CoA reductase	Isoform 2 only
	Q1HG43	Dual oxidase maturation factor 1	Isoforms 1 & 3 only
	Q6IA86	Elongator complex protein 2	Isoforms 1, 2, 3, 5, 6, & 7 only
	O15504	Nucleoporin-like protein 2	Isoform 2 only
	Q9UKA8	Calcipressin-3	Isoforms 1, 2, 5, & 6 only
CAP	P19419	ETS domain-containing protein Elk-1	Isoform 2 only
	Q6NT32	Carboxylesterase 5A	Isoforms 1, 2, 3, & 4 only
	P10914	Interferon regulatory factor 1	Isoform 1 only
	P15941	Mucin-1	Isoform 13 only
CAV	Q8N4H5	Mitochondrial import receptor subunit TOM5 homolog	Isoform 2 only
CDI	Q8N1A6	UPF0462 protein C4orf33	Isoform 1 only
	O95475	Homeobox protein SIX6	Isoform 1 only
CDP	O14990	Protein phosphatase inhibitor 2 family member C	Isoform 1 only
CDV	O95343	Homeobox protein SIX3	Isoform 1 only
CEF	Q8IWL1	Pulmonary surfactant-associated protein A2	Isoform 1 only
	Q8IWL2	Pulmonary surfactant-associated protein A1	Isoforms 1 & 2 only
	P35247	Pulmonary surfactant-associated protein D	Isoform 1 only
	P04155	Trefoil factor 1	Isoform 1 only
CEG	P78346	Ribonuclease P protein subunit p30	Isoform 1 only
	P0CG00	Putative zinc finger and SCAN domain-containing protein 5D	Isoform 1 only
CEV*	P56559	ADP-ribosylation factor-like protein 4C	Isoform 2 only
	Q7L8W6	Diphthine--ammonia ligase	Isoform 2 only
	Q86UE6	Leucine-rich repeat transmembrane neuronal protein 1	Isoform 1 only
	O43300	Leucine-rich repeat transmembrane neuronal protein 2	Isoform 1 only

	Q86VH5	Leucine-rich repeat transmembrane neuronal protein 3	Isoform 2 only
CEY	A1A4Y4	Immunity-related GTPase family M protein	Isoform 1 only
CFS	P31358	CAMPATH-1 antigen	Isoform 1 only
	Q86YW9	Mediator of RNA polymerase II transcription subunit 12-like protein	Isoforms 2 & 3 only
CFT*	Q8N957	Ankyrin repeat and fibronectin type-III domain-containing protein 1	Isoform 1 only
	Q13111	Chromatin assembly factor 1 subunit A	Isoform 2 only
	O15440	Multidrug resistance-associated protein 5	Isoform 2 only
CFV*	P05997	Collagen alpha-2(V) chain	Isoform 1 only
	Q96L33	Rho-related GTP-binding protein RhoV	Isoform 1 only
CGF	P0DML2	Chorionic somatomammotropin hormone 1	Isoform 1 only
	P0DML3	Chorionic somatomammotropin hormone 2	Isoforms 1 & 3 only
	Q14406	Chorionic somatomammotropin hormone-like 1	Isoforms 1, 2, 3, & 4 only
	Q13772	Nuclear receptor coactivator 4	Isoform 4 only
	P01242	Growth hormone variant	Isoforms 1 & 3 only
	P01241	Somatotropin	Isoforms 1, 2, 3, 4, & 5 only
CGI	Q8WVE0	EEF1A lysine methyltransferase 1	Isoform 1 only
	O15533	Tapasin	Isoform 2 only
CGV	Q15059	Bromodomain-containing protein 3	Isoform 2 only
	P61626	Lysozyme C	Isoform 1 only
	Q9BRL7	Vesicle-trafficking protein SEC22c	Isoform 1 only
CHF	O60548	Forkhead box protein D2	Isoform 1 only
CHP	Q9H3Y6	Tyrosine-protein kinase Srms	Isoform 1 only
CIE	Q96DP5	Methionyl-tRNA formyltransferase, mitochondrial	Isoform 1 only
	Q9Y6K9	NF-kappa-B essential modulator	Isoforms 1, 2, & 3 only
CII*	Q5T1H1	Protein eyes shut homolog	Isoform 2 only
	Q96CV9	Optineurin	Isoforms 1, 2, & 3 only
	Q6WBX8	Cell cycle checkpoint control protein RAD9B	Isoforms 1 & 4 only
	P82675	28S ribosomal protein S5, mitochondrial	Isoform 2 only
CIL	P08174	Complement decay-accelerating factor	Isoform 3 only
	Q13278	Putative protein RIG	Isoform 1 only
CIQ	Q96NS5	Ankyrin repeat and SOCS box protein 16	Isoform 1 only
	Q6ZVN8	Hemojuvelin	Isoform 1, 2, & 3 only
CKH	O14905	Protein Wnt-9b	Isoform 1 only

CKI	P26371	Keratin-associated protein 5-9	Isoform 1 only
	P61244	Protein max	Isoform 5 only
	Q7RTM1	Proton channel OTOP1	Isoform 1 only
CKK	O76000	Putative olfactory receptor 2B3	Isoform 1 only
	P25398	40S ribosomal protein S12	Isoform 1 only
	O43147	Small G protein signaling modulator 2	Isoform 5 only
	P04179	Superoxide dismutase [Mn], mitochondrial	Isoforms 1, 2, 3, & 4 only
CKP	P28335	5-hydroxytryptamine receptor 2C	Isoform 2 only
	Q15131	Cyclin-dependent kinase 10	Isoforms 1, 2, 3, & 7 only
CKS	P16671	Platelet glycoprotein 4	Isoform 2 only
	Q9NYP9	Protein Mis18-alpha	Isoform 1 only
	P08949	Neuromedin-B	Isoform 2 only
	Q9Y3N9	Olfactory receptor 2W1	Isoform 1 only
CKV	P30443	HLA class I histocompatibility antigen, A-1 alpha chain	Isoform 1 only
	P01892	HLA class I histocompatibility antigen, A-2 alpha chain	Isoform 1 only
	P04439	HLA class I histocompatibility antigen, A-3 alpha chain	Isoform 1 only
	P13746	HLA class I histocompatibility antigen, A-11 alpha chain	Isoforms 1 & 2 only
	P30447	HLA class I histocompatibility antigen, A-23 alpha chain	Isoform 1 only
	P05534	HLA class I histocompatibility antigen, A-24 alpha chain	Isoform 1 only
	P18462	HLA class I histocompatibility antigen, A-25 alpha chain	Isoform 1 only
	P30450	HLA class I histocompatibility antigen, A-26 alpha chain	Isoform 1 only
	P30512	HLA class I histocompatibility antigen, A-29 alpha chain	Isoform 1 only
	P16188	HLA class I histocompatibility antigen, A-30 alpha chain	Isoform 1 only
	P16189	HLA class I histocompatibility antigen, A-31 alpha chain	Isoform 1 only
	P10314	HLA class I histocompatibility antigen, A-32 alpha chain	Isoform 1 only
	P16190	HLA class I histocompatibility antigen, A-33 alpha chain	Isoform 1 only
	P30453	HLA class I histocompatibility antigen, A-34 alpha chain	Isoform 1 only
	P30455	HLA class I histocompatibility antigen, A-36 alpha chain	Isoform 1 only

	P30456	HLA class I histocompatibility antigen, A-43 alpha chain	Isoform 1 only
	P30457	HLA class I histocompatibility antigen, A-66 alpha chain	Isoform 1 only
	P01891	HLA class I histocompatibility antigen, A-68 alpha chain	Isoform 1 only
	P10316	HLA class I histocompatibility antigen, A-69 alpha chain	Isoform 1 only
	P30459	HLA class I histocompatibility antigen, A-74 alpha chain	Isoform 1 only
	Q09160	HLA class I histocompatibility antigen, A-80 alpha chain	Isoform 1 only
	Q9Y271	Cysteinyl leukotriene receptor 1	Isoform 1 only
	Q14680	Maternal embryonic leucine zipper kinase	Isoforms 1, 2, 3, 4, 5, 6, 7, & 8 only
CLD	Q8NBU5	ATPase family AAA domain-containing protein 1	Isoform 1 only
	Q9BSJ5	Uncharacterized protein C17orf80	Isoforms 1 & 2 only
	O95402	Mediator of RNA polymerase II transcription subunit 26	Isoform 1 only
	Q9UJV8	Purine-rich element-binding protein gamma	Isoform 1 only
	Q8WVN8	Ubiquitin-conjugating enzyme E2 Q2	Isoform 2 only
CLG	Q9UNE2	Rab effector Noc2	Isoforms 1 & 2 only
	Q9BU02	Thiamine-triphosphatase	Isoform 1 only
CLK	Q8IX21	SMC5-SMC6 complex localization factor protein 2	Isoform 2 only
	Q8TDI7	Transmembrane channel-like protein 2	Isoform 4 only
	P61081	NEDD8-conjugating enzyme Ubc12	Isoform 1 only
	Q86YA3	Protein ZGRF1	Isoform 3 only
CLL*	Q6ZUX7	LHFPL tetraspan subfamily member 2 protein	Isoform 1 only
	Q9NVC6	Mediator of RNA polymerase II transcription subunit 17	Isoform 1 only
	Q75NE6	Putative microRNA 17 host gene protein	Isoform 1 only
	Q00765	Receptor expression-enhancing protein 5	Isoform 2 only
	Q7Z2W9	39S ribosomal protein L21, mitochondrial	Isoforms 1 & 2 only
	Q96I59	Probable asparagine--tRNA ligase, mitochondrial	Isoforms 1 & 2 only
CLP	P02545	Prelamin-A/C	Isoform 4 only
	Q9Y5Y2	Cytosolic Fe-S cluster assembly factor NUBP2	Isoform 1 only
	Q9Y6I8	Peroxisomal membrane protein 4	Isoform 2 only
	Q96HL8	SH3 domain-containing YSC84-like protein 1	Isoform 5 only
	Q9P2F9	Zinc finger protein 319	Isoform 1 only
CLV*	Q9H694	Protein bicaudal C homolog 1	Isoform 2 only
	O60308	Centrosomal protein of 104 kDa	Isoform 2 only

	Q14166	Tubulin--tyrosine ligase-like protein 12	Isoform 1 only
CMF	Q6NVY1	3-hydroxyisobutyryl-CoA hydrolase, mitochondrial	Isoform 2 only
	Q9GZX9	Twisted gastrulation protein homolog 1	Isoform 1 only
CNL	Q9C091	GREB1-like protein	Isoform 4 only
	P29728	2'-5'-oligoadenylate synthase 2	Isoform 3 only
	P22234	Multifunctional protein ADE2	Isoforms 1 & 2 only
	Q9UJT1	Tubulin delta chain	Isoforms 1, 2, 3, 4, 5, & 6 only
CNR	O15194	CTD small phosphatase-like protein	Isoform 1 & 2 only
CPG	Q6ZMM2	ADAMTS-like protein 5	Isoform 1 & 2 only
	Q8TDQ1	CMRF35-like molecule 1	Isoform 2, 4, & 5 only
	Q8N9R0	Putative uncharacterized protein encoded by LINC00304	Isoform 1 only
	Q6TCH4	Membrane progestin receptor delta	Isoform 2 only
	Q9Y6S9	Ribosomal protein S6 kinase-like 1	Isoform 2 only
CPH	Q8NFI3	Cytosolic endo-beta-N-acetylglucosaminidase	Isoform 3 only
	Q9Y5X2	Sorting nexin-8	Isoform 1 only
CPI	Q9BSY4	Coiled-coil-helix-coiled-coil-helix domain-containing protein 5	Isoform 2 only
	Q9GZS3	WD repeat-containing protein 61	Isoform 1 only
CPK	Q9H9Q2	COP9 signalosome complex subunit 7b	Isoform 3 only
	Q3SYA9	Putative POM121-like protein 1	Isoform 1 only
	Q96LQ0	Protein phosphatase 1 regulatory subunit 36	Isoform 1 only
CPL	P30926	Neuronal acetylcholine receptor subunit beta-4	Isoform 2 only
	Q9UP79	A disintegrin and metalloproteinase with thrombospondin motifs 8	Isoform 1 only
	P38398	Breast cancer type 1 susceptibility protein	Isoform 2 only
	Q8TDM6	Disks large homolog 5	Isoforms 1, 2, 4, & 5 only
CPP	Q7Z695	Uncharacterized aarF domain-containing protein kinase 2	Isoform 1 only
	Q00975	Voltage-dependent N-type calcium channel subunit alpha-1B	Isoforms 2 only
	P56202	Cathepsin W	Isoform 1 only
	Q00535	Cyclin-dependent-like kinase 5	Isoform 1 & 2 only
	Q8NEG7	Protein DENND6B	Isoform 1 only
	O15527	N-glycosylase/DNA lyase	Isoform 2 only
	O75818	Ribonuclease P protein subunit p40	Isoforms 1 & 2 only
CPR	O95863	Zinc finger protein SNAI1	Isoform 1 only

	O43435	T-box transcription factor TBX1	Isoform 3 only
CPT	P25067	Collagen alpha-2(VIII) chain	Isoform 1 only
	Q99689	Fasciculation and elongation protein zeta-1	Isoform 1 only
	Q9UHY8	Fasciculation and elongation protein zeta-2	Isoforms 1 & 2 only
	Q93038	Tumor necrosis factor receptor superfamily member 25	Isoforms 5 & 6 only
CPV	P60981	Destrin	Isoforms 1 & 2 only
	P55265	Double-stranded RNA-specific adenosine deaminase	Isoforms 1, 2, 3, 4, & 5 only
	Q7Z7L9	Zinc finger and SCAN domain-containing protein 2	Isoform 4 only
CPY	P51460	Insulin-like 3	Isoform 1 only
CQA	Q92784	Zinc finger protein DPF3	Isoform 1 only
	Q5VZR4	Hippocampus abundant transcript-like protein 2	Isoform 1 only
	P09086	POU domain, class 2, transcription factor 2	Isoform 4 only
CQL*	Q8WXX0	Dynein heavy chain 7, axonemal	Isoform 4 only
	P55082	Microfibril-associated glycoprotein 3	Isoforms 1 & 2 only
CQS	P04054	Phospholipase A2	Isoform 1 only
	A6NFN3	RNA binding protein fox-1 homolog 3	Isoform 2 only
CQV	Q96M34	Testis-specific expressed protein 55	Isoform 1 only
	P0C0L4	Complement C4-A	Isoforms 1 & 2 only
	P0C0L5	Complement C4-B	Isoform 1 only
	Q8IWL8	Saitohin	Isoform 1 only
CRD	Q86UQ0	Zinc finger protein 589	Isoforms 1 & 2 only
CRE	Q96CX2	BTB/POZ domain-containing protein KCTD12	Isoform 1 only
	Q15615	Olfactory receptor 4D1	Isoform 1 only
CRF	P09466	Glycodelin	Isoforms 1, 2, & 3 only
CRL	Q8TDW5	Synaptotagmin-like protein 5	Isoforms 1 & 2 only
CRQ	P08908	5-hydroxytryptamine receptor 1A	Isoform 1 only
	Q6P2C8	Mediator of RNA polymerase II transcription subunit 27	Isoforms 1 & 2 only
	Q4G0F5	Vacuolar protein sorting-associated protein 26B	Isoform 1 only
CRV	Q9Y2C4	Nuclease EXOG, mitochondrial	Isoform 2 only
CSA	Q1MX18	Protein inscuteable homolog	Isoform 6 only
	Q8N983	39S ribosomal protein L43, mitochondrial	Isoform 1 only
	P51811	Membrane transport protein XK	Isoform 1 only
CSF	Q9BTY2	Plasma alpha-L-fucosidase	Isoform 2 only

	Q6QHF9	Peroxisomal N(1)-acetyl-spermine/spermidine oxidase	Isoform 8 only
	Q9Y2Y8	Proteoglycan 3	Isoform 1 only
	P12757	Ski-like protein	Isoform 2 only
CSG	P41223	Protein BUD31 homolog	Isoform 1 only
	O43866	CD5 antigen-like	Isoform 1 only
	Q96KN8	Phospholipase A and acyltransferase 5	Isoform 2 only
	Q12794	Hyaluronidase-1	Isoform 4 only
	Q9BXT6	RNA helicase Mov10l1	Isoform 5 only
CSI	Q9Y2T5	G-protein coupled receptor 52	Isoform 1 only
	Q9Y5Q9	General transcription factor 3C polypeptide 3	Isoform 1 only
CSK	Q53EZ4	Centrosomal protein of 55 kDa	Isoform 1 only
	P04275	von Willebrand factor	Isoform 1 only
CSL	Q9P2M7	Cingulin	Isoform 2 only
	O95278	Laforin	Isoforms 1, 3, 6, 7, & 8 only
	O75593	Forkhead box protein H1	Isoform 1 only
	Q6KB66	Keratin, type II cytoskeletal 80	Isoform 2 only
	P14598	Neutrophil cytosol factor 1	Isoform 2 only
	Q9NQB0	Transcription factor 7-like 2	Isoform 12 only
	Q96N03	V-set and transmembrane domain-containing protein 2-like protein	Isoform 1 only
	Q9ULD5	Zinc finger protein 777	Isoform 1 only
CSQ	Q5HY92	Fidgetin	Isoform 1 only
	Q8IVF1	NUT family member 2A	Isoform 1 only
	A6NNL0	NUT family member 2B	Isoform 1 only
	Q5VT03	NUT family member 2D	Isoform 1 only
	B1AL46	NUT family member 2E	Isoform 1 only
	A1L443	NUT family member 2F	Isoform 1 only
	Q5VZR2	NUT family member 2G	Isoform 1 only
	P33764	Protein S100-A3	Isoform 1 only
CSS	Q5T1V6	Probable ATP-dependent RNA helicase DDX59	Isoform 2 only
	Q9H0R5	Guanylate-binding protein 3	Isoform 4 only
	P05112	Interleukin-4	Isoforms 1 & 2 only
	Q86X10	Ral GTPase-activating protein subunit beta	Isoforms 1, 2, 3, & 4 only
	Q9NY57	Serine/threonine-protein kinase 32B	Isoforms 1 & 2 only
CST	Q9BPY3	Protein FAM118B	Isoform 1 only
	Q8TC05	Nuclear protein MDM1	Isoform 3 only
	P16471	Prolactin receptor	Isoform 9 only
	Q7Z4G4	tRNA (guanine(10)-N2)-methyltransferase homolog	Isoform 3 only

CSV*	Q9UEW3	Macrophage receptor MARCO	Isoforms 1 & 2 only
	Q9H3H1	tRNA dimethylallyltransferase	Isoforms 1, 2, 3, 4, 5, & 6 only
	A8MW92	PHD finger protein 20-like protein 1	Isoform 1 only
	Q9UKA9	Polypyrimidine tract-binding protein 2	Isoforms 5 & 6 only
	O95969	Secretoglobin family 1D member 2	Isoform 1 only
CSY	P13727	Bone marrow proteoglycan	Isoforms 1 & 2 only
	A4D1P6	WD repeat-containing protein 91	Isoform 3 only
CTE	Q9H3K6	BolA-like protein 2	Isoform 2 only
	Q14204	Cytoplasmic dynein 1 heavy chain 1	Isoform 1 only
	Q14141	Septin-6	Isoform 1 only
	Q6PF04	Zinc finger protein 613	Isoforms 1 & 2 only
CTH	P02790	Hemopexin	Isoform 1 only
CTP	Q9UBL6	Copine-7	Isoforms 1 & 2 only
	Q5T1A1	DC-STAMP domain-containing protein 2	Isoform 2 only
	O43776	Asparagine--tRNA ligase, cytoplasmic	Isoform 1 only
CTS	P28222	5-hydroxytryptamine receptor 1B	Isoform 1 only
	Q6UX04	Spliceosome-associated protein CWC27 homolog	Isoform 2 only
	O15534	Period circadian protein homolog 1	Isoform 1 only
CTV*	A6H8Y1	Transcription factor TFIIIB component B" homolog	Isoform 2 only
	O75044	SLIT-ROBO Rho GTPase-activating protein 2	Isoform 1 only
CVF	P59541	Taste receptor type 2 member 30	Isoform 1 only
	P29144	Tripeptidyl-peptidase 2	Isoform 1 only
CVG	Q96PK6	RNA-binding protein 14	Isoform 3 only
	Q9BWF3	RNA-binding protein 4	Isoform 3 only
	Q9Y336	Sialic acid-binding Ig-like lectin 9	Isoform 2 only
CVK	P01008	Antithrombin-III	Isoform 1 only
	Q9C0E2	Exportin-4	Isoform 1 only
CVL*	Q32MH5	Protein FAM214A	Isoform 2 only
	P23510	Tumor necrosis factor ligand superfamily member 4	Isoforms 1 & 2 only
CVM	Q12946	Forkhead box protein F1	Isoform 1 only
	Q12947	Forkhead box protein F2	Isoform 1 only
	Q9NS66	Probable G-protein coupled receptor 173	Isoform 1 only
CVY	Q14156	Protein EFR3 homolog A	Isoforms 1, 2, & 3 only
	Q9Y2G0	Protein EFR3 homolog B	Isoforms 1 & 2 only
	Q6ZUU3	FOXL2 neighbor protein	Isoform 1 only

	Q92750	Transcription initiation factor TFIID subunit 4B	Isoform 2 only
CWF	Q9UF11	Pleckstrin homology domain-containing family B member 1	Isoforms 1, 2, 3, & 4 only
CWI	Q68EM7	Rho GTPase-activating protein 17	Isoform 5 only
CYL	Q9BQ51	Programmed cell death 1 ligand 2	Isoform 3 only

*sequences that were also recovered from yeast genetic screening

Table S5. Integrations of FPP/GGPP competition experiments from two independent trials.

Peptide	GGPP:FPP molar ratio	Substrate	Farnesylated product	Geranylgeranylated product	Percent farnesylated	Percent geranylgeranylated
dns-GCFT	100%:0%	0.186	0	4.014	0	95.6
	75%:25%	0.120	2.251	0.857	69.7	26.6
	50%:50%	0.065	3.176	0.051	96.5	1.6
	25%:75%	0.061	3.626	0	98.3	0
	0%:100%	0.071	3.639	0	98.1	0
dns-GCFV						
	100%:0%	0.019	0.044	1.186	3.5	94.9
	75%:25%	0	0.769	0.115	86.9	13.0
	50%:50%	0	0.764	0.039	95.1	4.9
	25%:75%	0	0.822	0.011	98.7	1.3
	0%:100%	0	0.932	0	100	0
dns-GCGF						
	100%:0%	0.066	0.067	0.371	13.2	73.6
	75%:25%	0.057	0.386	0	87.1	0
	50%:50%	0.047	0.349	0	88.2	0
	25%:75%	0.043	0.391	0	90.1	0
	0%:100%	0.050	0.380	0	88.3	0
dns-GCMF						
	100%:0%	0.010	0.049	0.474	9.3	88.7
	75%:25%	0	0.496	0.029	94.4	5.6
	50%:50%	0.013	0.485	0.025	92.7	4.8
	25%:75%	0	0.450	0	100	0
	0%:100%	0.016	0.360	0	95.7	0
dns-GCVF						
	100%:0%	0.026	0.035	0.525	6.1	89.5
	75%:25%	0.040	0.263	0.022	80.9	6.8
	50%:50%	0.043	0.278	0	86.7	0
	25%:75%	0.047	0.278	0	85.7	0
	0%:100%	0.044	0.234	0	84.1	0

Peptide	GGPP:FPP molar ratio	Substrate	Farnesylated product	Geranylgeranylated product	Percent farnesylated	Percent geranylgeranylated
dns-GCFT	100%:0%	0.206	0.084	3.725	2.1	92.8
	75%:25%	0.116	2.046	1.037	63.9	32.4
	50%:50%	0.061	3.091	0.066	96.0	2.1
	25%:75%	0.028	3.622	0.026	98.5	0.7
	0%:100%	0.062	3.272	0	98.1	0
dns-GCFV						
	100%:0%	0	0.026	1.267	2.0	97.9
	75%:25%	0	0.737	0.121	85.9	14.1
	50%:50%	0	0.787	0.035	95.7	4.3
	25%:75%	0	0.799	0	100	0
	0%:100%	0	0.923	0	100	0
dns-GCGF						
	100%:0%	0.035	0.043	0.339	10.4	81.1
	75%:25%	0.036	0.388	0	91.5	0
	50%:50%	0.038	0.417	0	91.7	0
	25%:75%	0.033	0.382	0	92.1	0
	0%:100%	0.037	0.413	0	91.9	0
dns-GCMF						
	100%:0%	0.019	0.026	0.459	5.6	90.7
	75%:25%	0	0.448	0.033	93.2	6.8
	50%:50%	0	0.425	0	100	0
	25%:75%	0.019	0.402	0	95.5	0
	0%:100%	0	0.477	0	100	0
dns-GCVF						
	100%:0%	0.019	0.019	0.588	3.1	93.8
	75%:25%	0.032	0.259	0.057	74.4	16.4
	50%:50%	0.034	0.228	0.013	82.8	4.8
	25%:75%	0.065	0.244	0	79.0	0
	0%:100%	0.042	0.293	0	87.3	0

Table S6. Potential FTase substrate sequences within the human proteome

Potential FTase substrate sequences	Prosite search string[b]	Number of sequences
unconstrained searches		
Cxx	Cxx>	1074
CaaX	Cxxx>	1208
$C(x)_3X$	Cxxxx>	1015
constrained searches[a]		
Cxx not within CaaX or $C(x)_3X$	{c}{c}C{c}{c}>	847
CaaX without downstream Cys	C{c}{c}{c}>	1062
$C(x)_3X$ without downstream Cys	C{c}{c}{c}{c}>	824

[a]noted constraints were applied to ignore Cxx sequences that were part of a longer sequence (e.g. CCxx and CxCxx) and potential GGTase-II consensus sequences (e.g. CxC, xCC).

[b]lower case c denotes positions where Cys was a negative constraint; > denotes the C-terminal constraint.

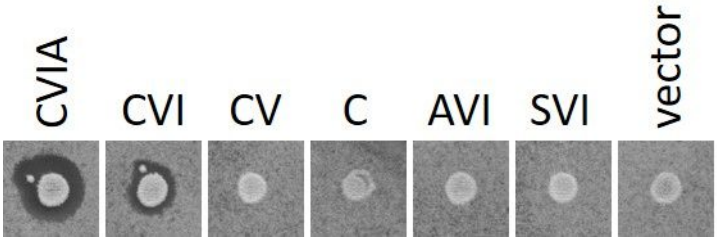

Figure S1. Phenotypes of a-factor CaaX mutants. The indicated plasmid-encoded **a**-factor mutants were expressed in *MAT***a** *mfa1 mfa2* yeast (SM2331) and evaluated by the halo assay as described in Figure 1. Vector is the parent plasmid lacking the *MFA1* gene.

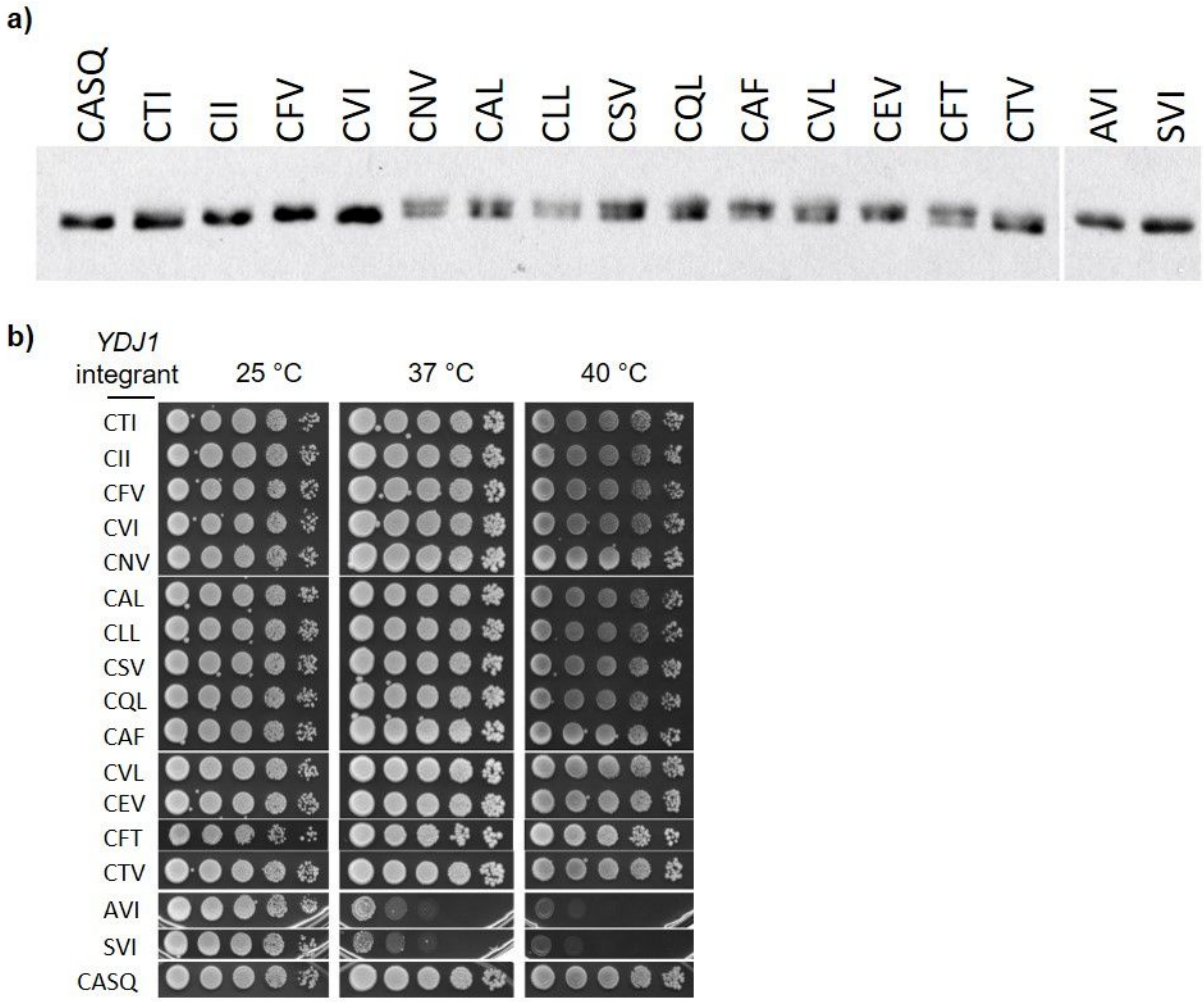

Figure S2. Gel-shift and thermotolerance patterns of integrated Ydj1p Cxx variants. The indicated Ydj1p Cxx variants were assessed for farnesylation by a) the mobility shift and b) thermotolerance assays as described in Figure 2b except that extracts were prepared from strains containing an integrated copy of the indicated Ydj1p Cxx variant rather than being plasmid-encoded. Panel a shows a representative example of one of the three technical replicates that was used to quantify the extent of farnesylation. The upper and lower bands represent unfarnesylated and farnesylated Ydj1p, respectively. Panel b shows a representative example of one of two technical replicates.

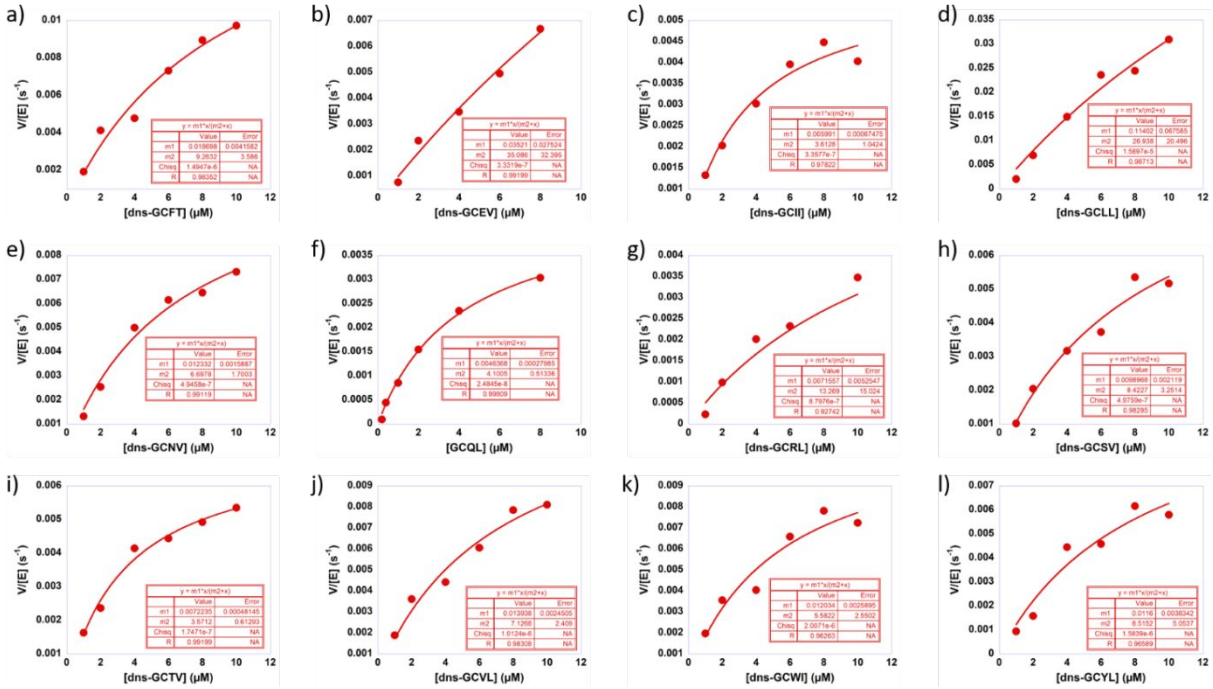

Figure S3. Steady state characterization of FTase-catalyzed dns-GCxx peptide farnesylation with FPP. a-l) Representative plots for dependence of farnesylation activity on peptide substrate concentration catalyzed by FTase in the presence of 10 μM FPP: a) dns-GCFT; b) dns-GCEV; c) dns-GCII; d) dns-GCLL; e) dns-GCNV; f) dns-GCQL; g) dns-GCRL; h) dns-GCSV; i) dns-GCTV; j) dns-GCVL; k) dns-GCWI; l) dns-GCYL. The curve represents the best fit to the Michaelis-Menten equation. Reactions were performed and analyzed as described in Materials and Methods.

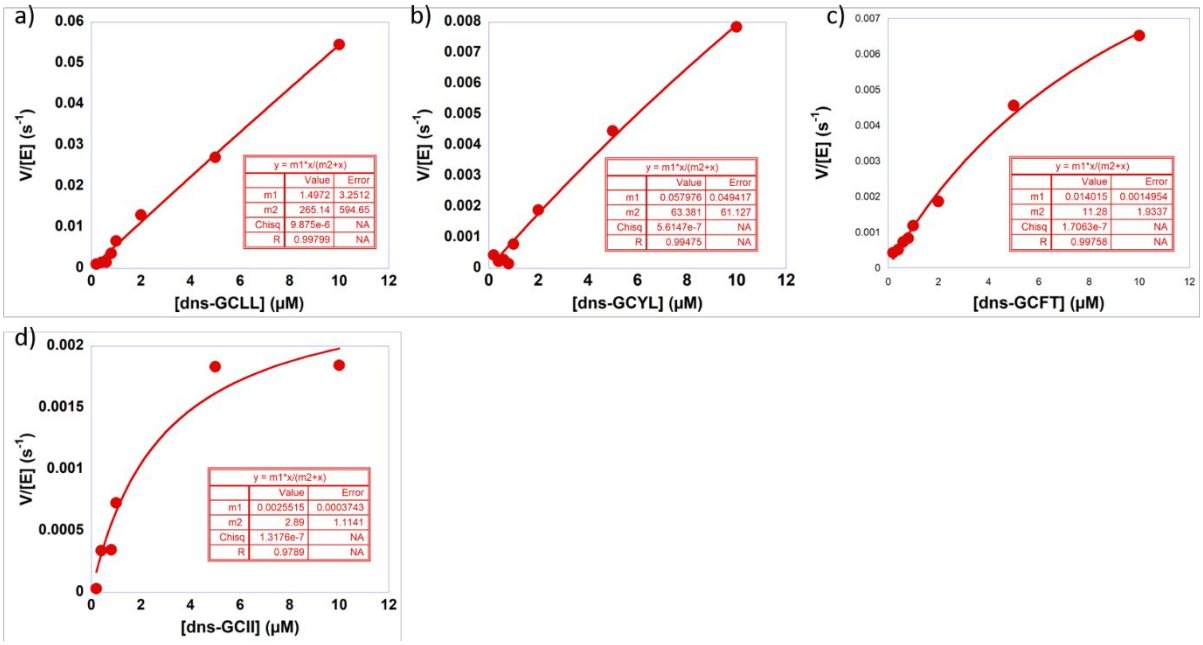

Figure S4. Steady state characterization of FTase-catalyzed dns-GCxx peptide geranylgeranylation with GGPP. a-d) Representative plots for dependence of farnesylation activity on peptide substrate concentration catalyzed by FTase in the presence of 10 μM GGPP: a) dns-GCLL; b) dns-GCYL; c) dns-GCFT; d) dns-GCII. The curve represents the best fit to the Michaelis-Menten equation. Reactions were performed and analyzed as described in Materials and Methods.

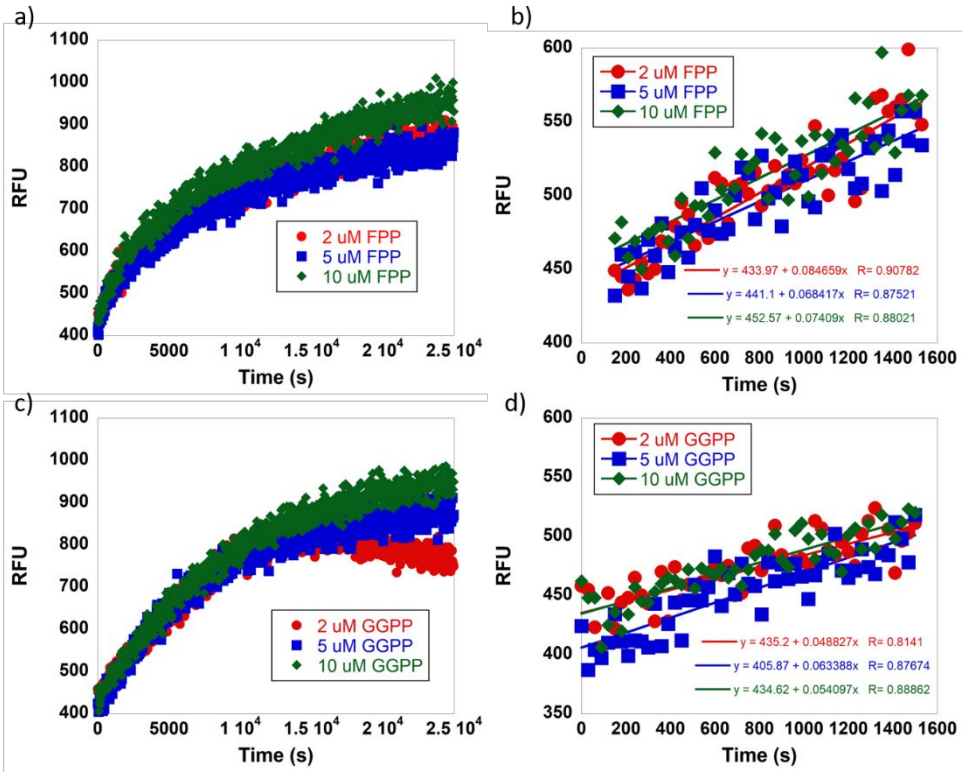

Figure S5. Determination of saturating FPP and GGPP concentrations for FTase-catalyzed prenylation of dns-GCFT. a) Time course for FTase-catalyzed farnesylation of dns-GCFT in the presence of various FPP concentrations (2 μM, red; 5 μM, blue; 10 μM, green); b) Determination of initial velocities for FTase-catalyzed farnesylation of dns-GCFT in the presence of various FPP concentrations (2 μM, red; 5 μM, blue; 10 μM, green). c) Time course for FTase-catalyzed geranylgeranylation of dns-GCFT in the presence of various GGPP concentrations (2 μM, red; 5 μM, blue; 10 μM, green); b) Determination of initial velocities for FTase-catalyzed geranylgeranylation of dns-GCFT in the presence of various GGPP concentrations (2 μM, red; 5 μM, blue; 10 μM, green). Reactions were performed and analyzed as described in Materials and Methods.

Supporting References

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