Supporting Information

Peptide Nanofiber Complexes with siRNA for Deep Brain Gene Silencing by Stereotactic Neurosurgery

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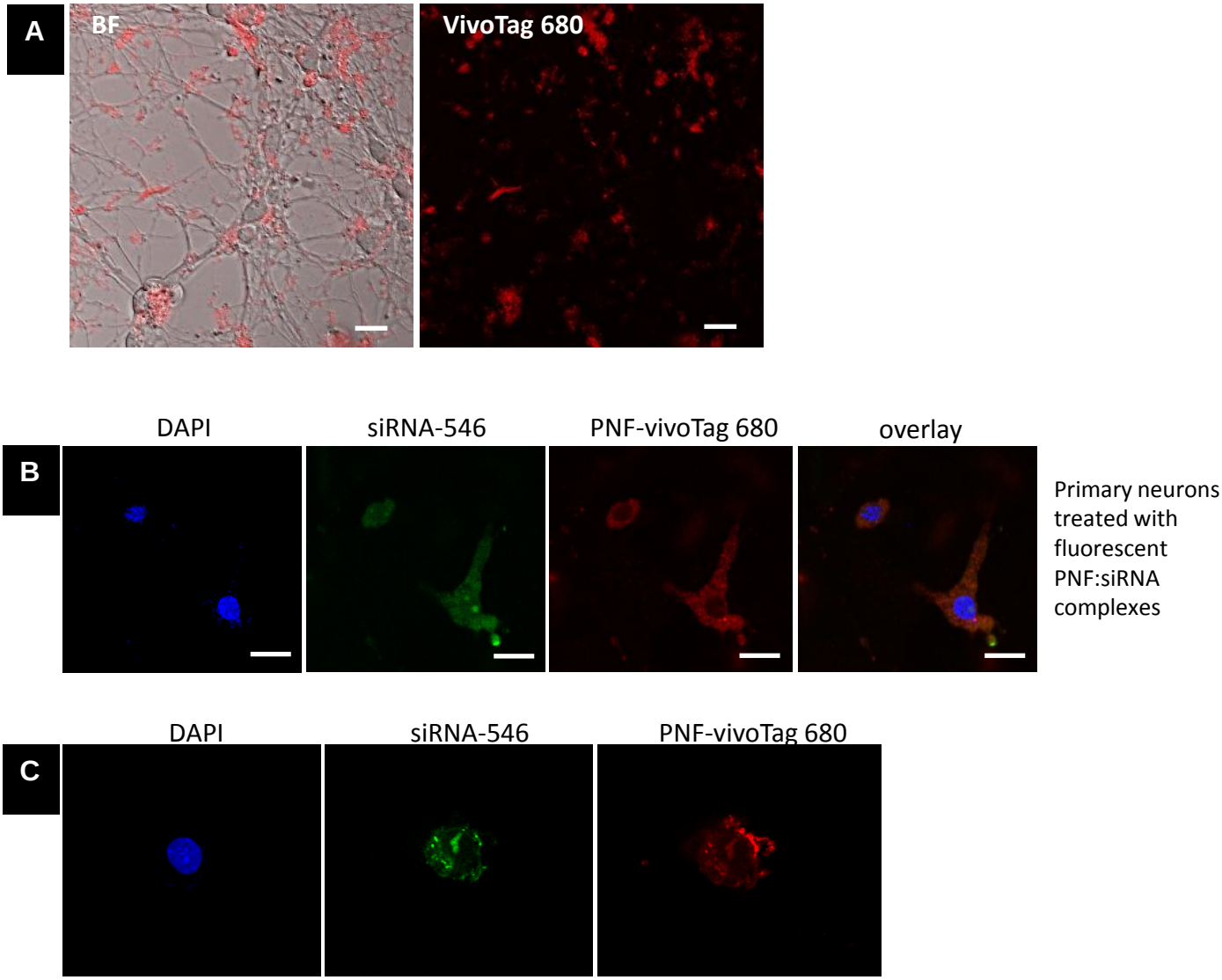
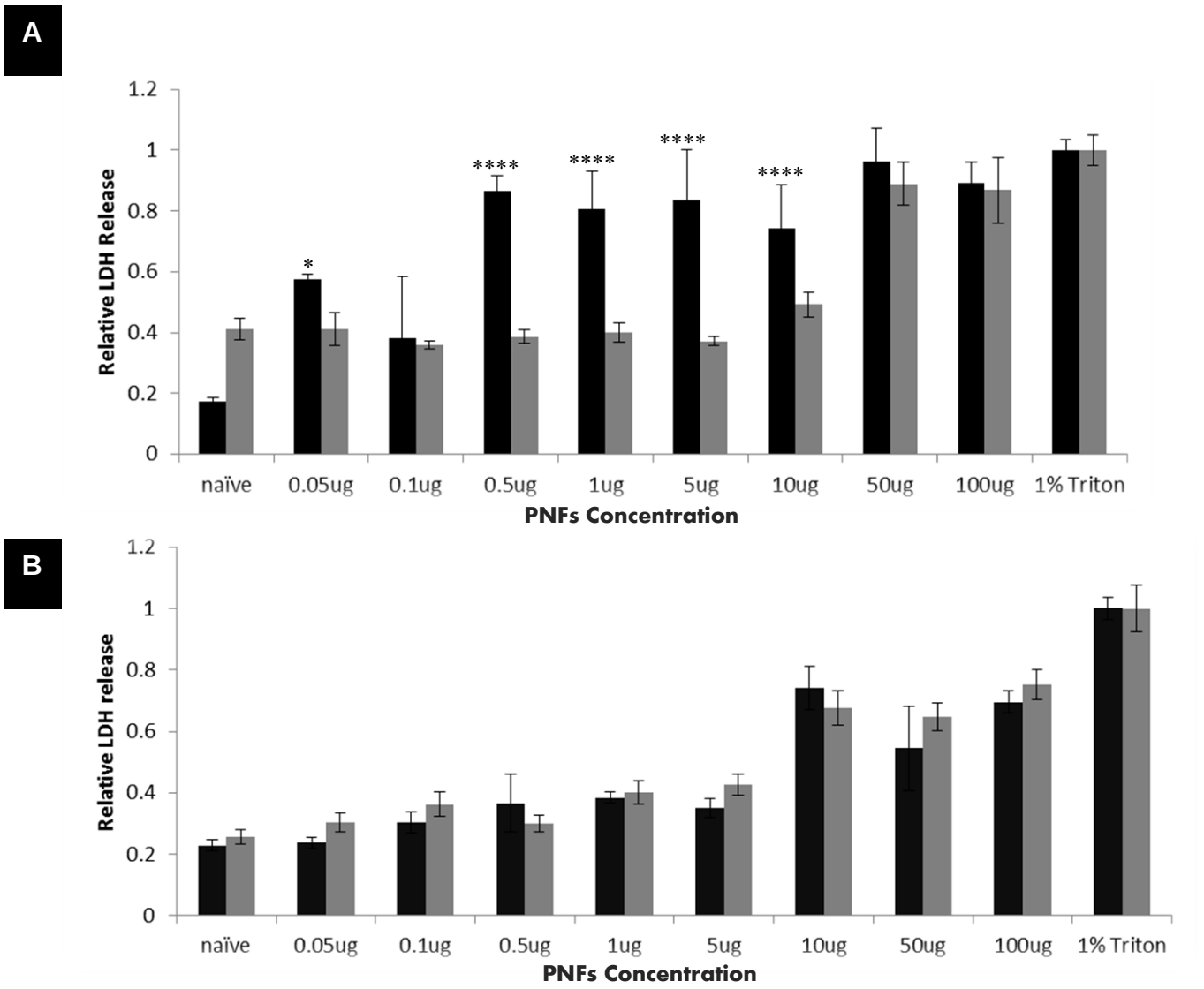


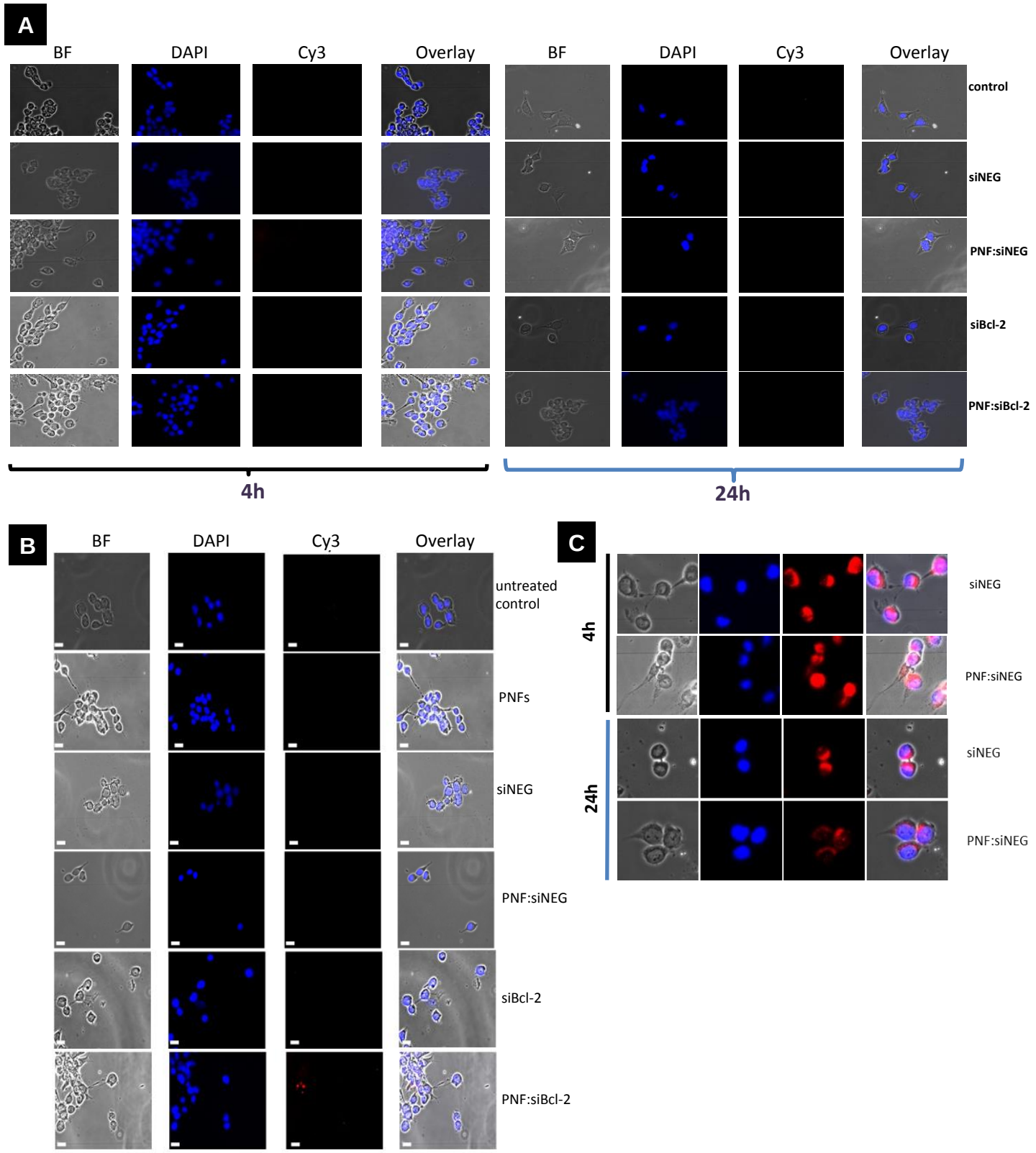
Supporting Figure 1: Cellular internalization of PNF:siRNA. (A) Confocal laser scanning microscopy pictures of primary rat neurons treated with fluorescently labelled PNFs (BF = bright field; Scale bar = 20 microns). **(B)** PNFs were covalently tagged with Vivo-Tag 680 XL (red) and siRNA was labelled with Alexa 546 (green). Cell nuclei were stained with DAPI (blue). 20X magnification; Scale bar = 10 microns) . **(C)** unmerged channels of SH-SY5Y cell incubated with fluorescently-labelled PNF:siRNA complexes.

Supporting Figure 1



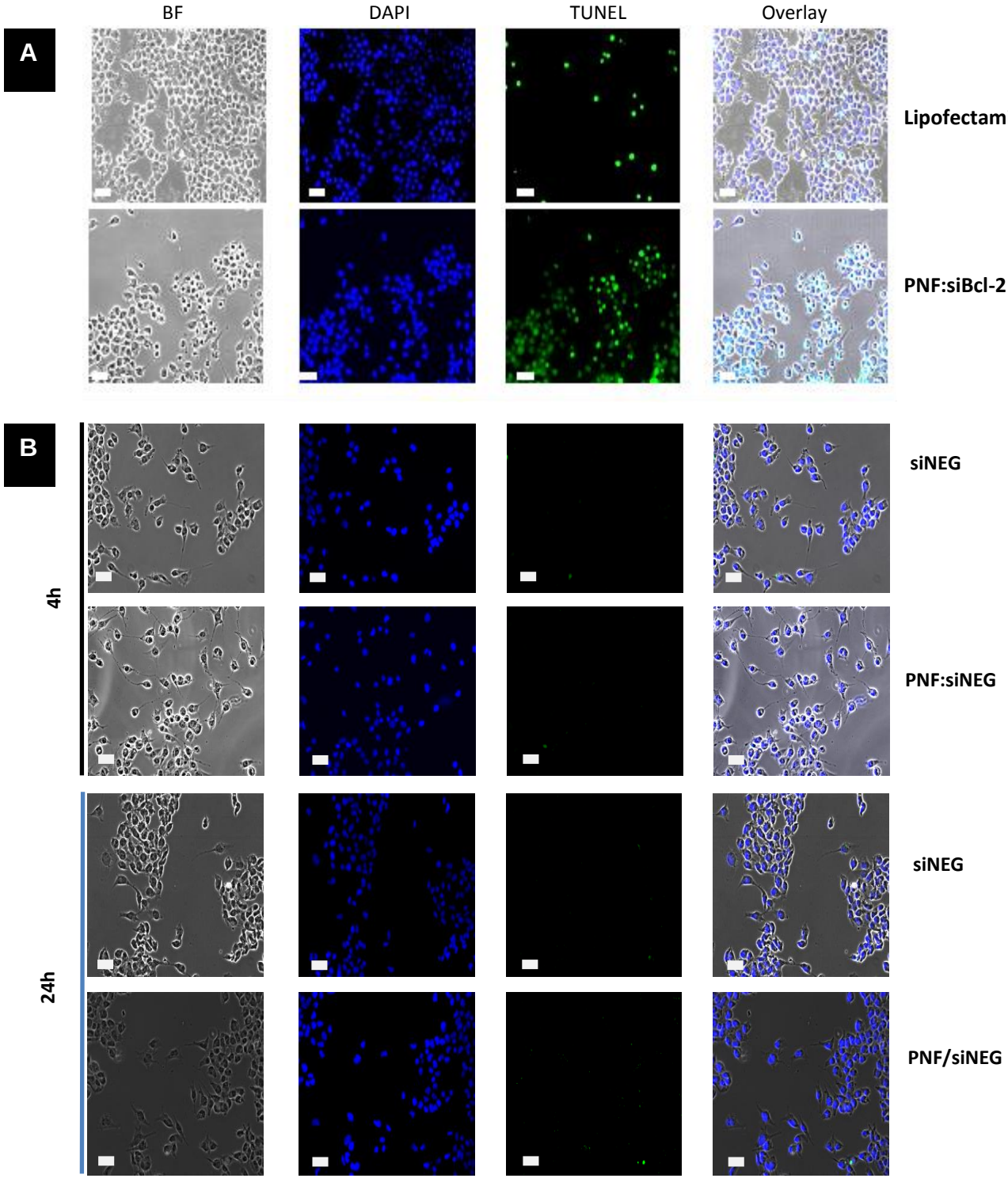
Supporting Figure 2: Cytotoxicity of PNFs as function of concentration on SH-SY5Y cells. (A) LDH release normalised to 1% Triton –treated cells, exposed to increasing concentration (μg/mL) of PNFs after 4h incubation in serum-free media (black column) and 4 hr incubation in serum-free media followed 20h recovery in FBS (grey column). **(B)** LDH release normalised to 1% Triton –treated cells exposed to increasing concentration (μg/mL) of PNFs in serum supplemented (FBS) media after 4h (black column) and 24h (grey column) (Two-way ANOVA, $P<0.0001$).

Supporting Figure 2



Supporting Figure 3: Immunofluorescence assessment of Bcl-2 knockdown. (A) Epi-fluorescence images of human SH-SY5Y 4h and 24h after transfection. The specificity of the Bcl-2 antibody was assessed by treating the transfected cells with the secondary antibody only tagged with Cy3. There was no detactable fluorescence in the Cy3 channel, confirming the Bcl-2 antibody specificity. **(B)** Immunofluorescence with caspase-3 specific antibody 4h post-transfection. Only cells treated with PNF/siBcl2 showed expression of caspase-3 (red fluorescence; BF=bright field). **(C)** Bcl2 levels after gene silencing in SH-SY5Y cells with siNEG and PNF:siNEG control treatment groups.

Supporting Figure 3



Supporting Figure 4: (A) Epi-fluorescence microscopy images of TUNEL staining on transfected SH-SY5Ycells for the detection of apoptosis**.** 4 hours after transfection more cells undergo apoptosis with the PNF:siBcl-2 treatment in comparison to the conventional transfection agent Lipofectamine (Lipofectamine:siBcl-2). Magnification 20X; Scale bar is 40 microns; BF= bright field). **(B)** Epi-fluorescence microscopy images of TUNEL staining on transfected SH-SY5Ycells with siNEG and PNF:siNEG control treatment groups. Of note there is some background green fluorescence.

Supporting Figure 4