A

Human Notch core binding domain alignment

```
                10        20        30        40        50        60        70        80        90       100
N1 10-14 >  ARGPRCISNPCNEGSNCDTNPVNGKAICTCPSGYTGPACSQDVDECSLG---ANPCEHAGKCINTLGSFECQCLQGYTGPRCEIDVNECVSNPCQNDATC  97
N2 10-14 >  -LDDACISNPCHKGALCDTNPLNGQYICTCPQGYKGADCT-DVDECAMAN--SNPCEHAGKCVNTDGAFHCECLKGYAGPRCE-DINECHSDPCQNDATC  95
N3 10-14 >  ----------------------------------------DVDECSIG---ANPCEHLGRCVNTQGSFLCQCGRGYTGPRCE-DVNECLSGPCRNQATC  55
N4 10-14 >  -LEDMCLSQPCHGDAQCSTNPLTGSTLCLCQPGYSGPTCHQDLDECLMAQQGPSPCEHGGSCLNTPGSFNCLCPPGYTGSRCEADHNECLSQPCHPGSTC  99

                110       120       130       140       150       160       170       180       190       200
N1 10-14 >  LDQIGEFQCICMPGYEGVHCEVNTDECASSPCLHNGRCLDKINEFQCECPTGFTGHLCQYDVDECASTPCKNGAKCLDGPNTYTCVCTEGYTGTHCE---  194
N2 10-14 >  LDKIGGFTCLCMPGFKGVHCE-EINECQSNPCVNNGQCVDKVNRFQCLCPPGFTGPVCQ-DIDDCSSTPCLNGAKCIDHPNGYECQCATGFTGVLCE---  190
N3 10-14 >  LDRIGQFTCICMAGFTGTYCE-DIDECQSSPCVNGGVCKDRVNGFSCTCPSGFSGSTCQ-DVDECASTPCRNGAKCVDQPDGYECRCAEGFEGTLCDNVD  153
N4 10-14 >  LDLLATFHCLCPPGLEGQLCEVETNECASAPCLNHADCHDLLNGFQCICLPGFSGTRCEEDIDECRSSPCANGGQCQDQPGAFHCKCLPGFEGPRCQ---  196

                210       220       230
N1 10-14 >  ----------------------------------  194
N2 10-14 >  ----------------------------------  190
N3 10-14 >  DCSPDPCHHGRCVDGIASFSCACAPGYTGTRCE  186
N4 10-14 >  ----------------------------------  196
```

B

Human/mouse Notch core binding domain alignment

```
                10        20        30        40        50        60        70        80        90       100
hN1 10-14 >  ARGPRCISNPCNEGSNCDTNPVNGKAICTCPSGYTGPACSQDVDECSLGANPCEHAGKCINTLGSFECQCLQGYTGPRCEIDVNECVSNPCQNDATCLDQ  100
mN1 10-14 >  L-NDA.....................................-.....A.............L.....................-.....I.............  97

                110       120       130       140       150       160       170       180       190
hN1 10-14 >  IGEFQCICMPGYEGVHCEVNTDECASSPCLHNGRCLDKINEFQCECPTGFTGHLCQYDVDECASTPCKNGAKCLDGPNTYTCVCTEGYTGTHCE  194
mN1 10-14 >  ..............Y..-..............H.M........Q..K..N.....-.....................................  189
```

```
                10        20        30        40        50        60        70        80        90       100
hN2 10-14 >  LDDACISNPCHKGALCDTNPLNGQYICTCPQGYKGADCTDVDECAMANSNPCEHAGKCVNTDGAFHCECLKGYAGPRCEDINECHSDPCQNDATCLDKIG  100
mN2 10-14 >  ..........................................................................................  100

                110       120       130       140       150       160       170       180       190
hN2 10-14 >  GFTCLCMPGFKGVHCEEINECQSNPCVNNGQCVDKVNRFQCLCPPGFTGPVCQDIDDCSSTPCLNGAKCIDHPNGYECQCATGFTGVLCE  190
mN2 10-14 >  ..................V.....................................................I..D  190
```

```
                10        20        30        40        50        60        70        80        90       100
hN3 10-14 >  DVDECSIGANPCEHLGRCVNTQGSFLCQCGRGYTGPRCEDVNECLSGPCRNQATCLDRIGQFTCICMAGFTGTYCEDIDECQSSPCVNGGVCKDRVNGFS  100
mN3 10-14 >  ..........................................................................................  100

                110       120       130       140       150       160       170       180
hN3 10-14 >  CTCPSGFSGSTCQDVDECASTPCRNGAKCVDQPDGYECRCAEGFEGTLCDNVDDCSPDPCHHGRCVDGIASFSCACAPGYTGTRCE  186
mN3 10-14 >  ..........M.....................................E.....................I...  186
```

```
                10        20        30        40        50        60        70        80        90       100
hN4 10-14 >  LEDMCLSQPCHGDAQCSTNPLTGSTLCLCQPGYSGPTCHQDLDECLMAQQGPSPCEHGGSCLNTPGSFNCLCPPGYTGSRCEADHNECLSQPCHPGSTCL  100
mN4 10-14 >  ..........VN..............I.......S...-.....Q..............I..........L.........-..................  98

                110       120       130       140       150       160       170       180       190
hN4 10-14 >  DLLATFHCLCPPGLEGQLCEVETNECASAPCLNHADCHDLLNGFQCICLPGFSGTRCEEDIDECRSSPCANGGQCQDQPGAFHCKCLPGFEGPRCQ  196
mN4 10-14 >  ..............R...-.V...T.N....Q.A..........L.....T.A..-..M...S.T......R.R......Y.E........H.E  192
```

Figure S1: Comparison of Notch putative core binding domains within and between species. (A) Amino acid alignment of EGFs 10-14 of the four human Notch receptors. Pink highlighting indicates identity, dashes indicate lack of alignment. (B) Amino acid alignment of EGFs 10-14 of each human Notch protein with its murine ortholog. Dots indicate amino acid identity.

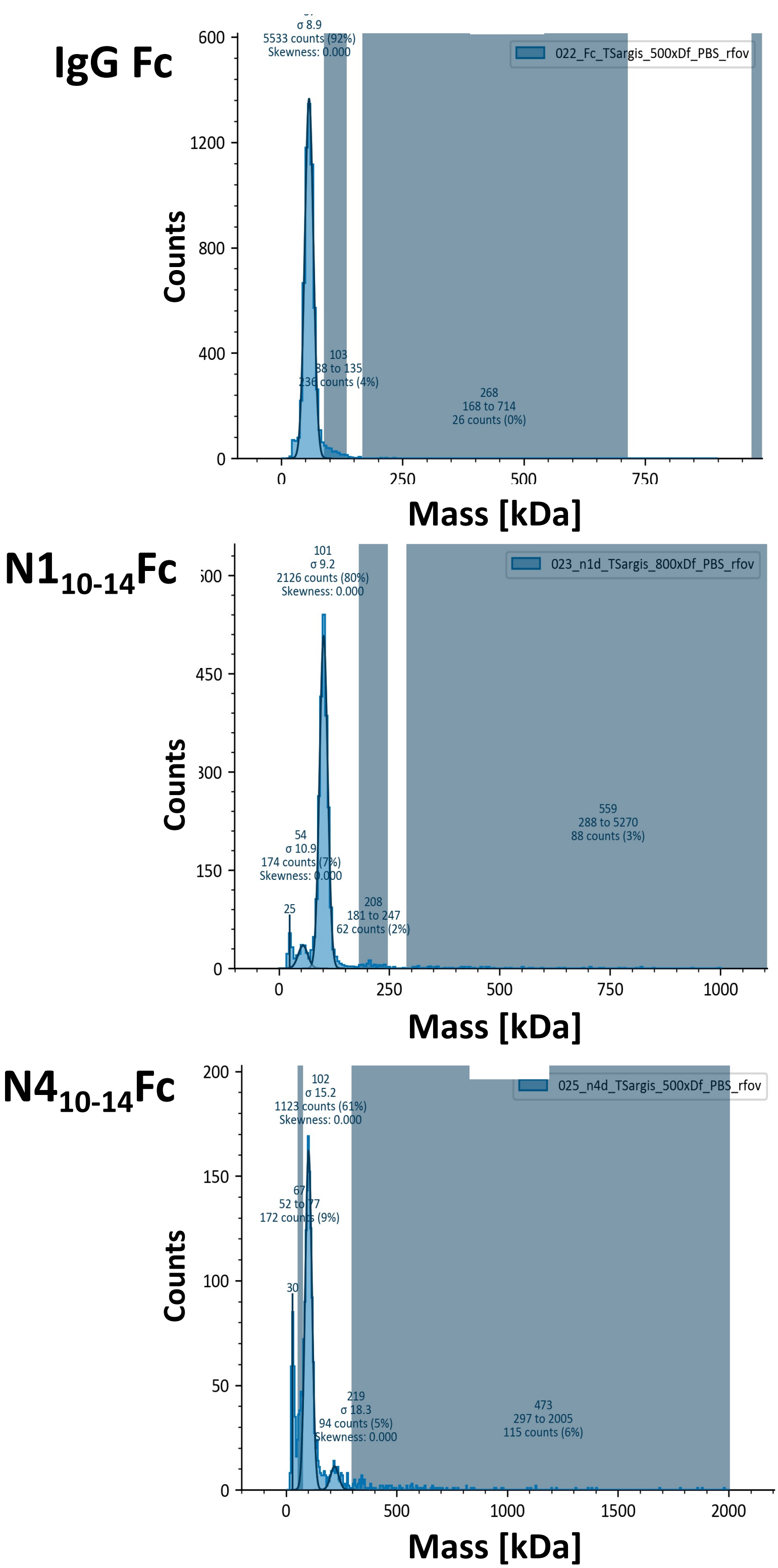
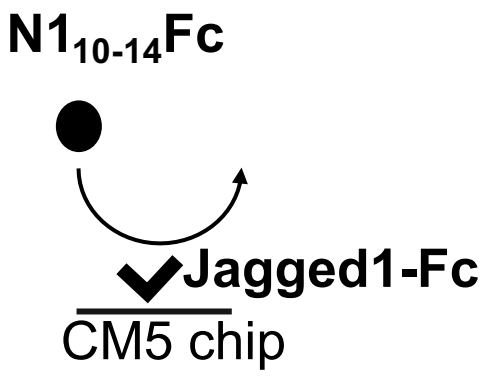
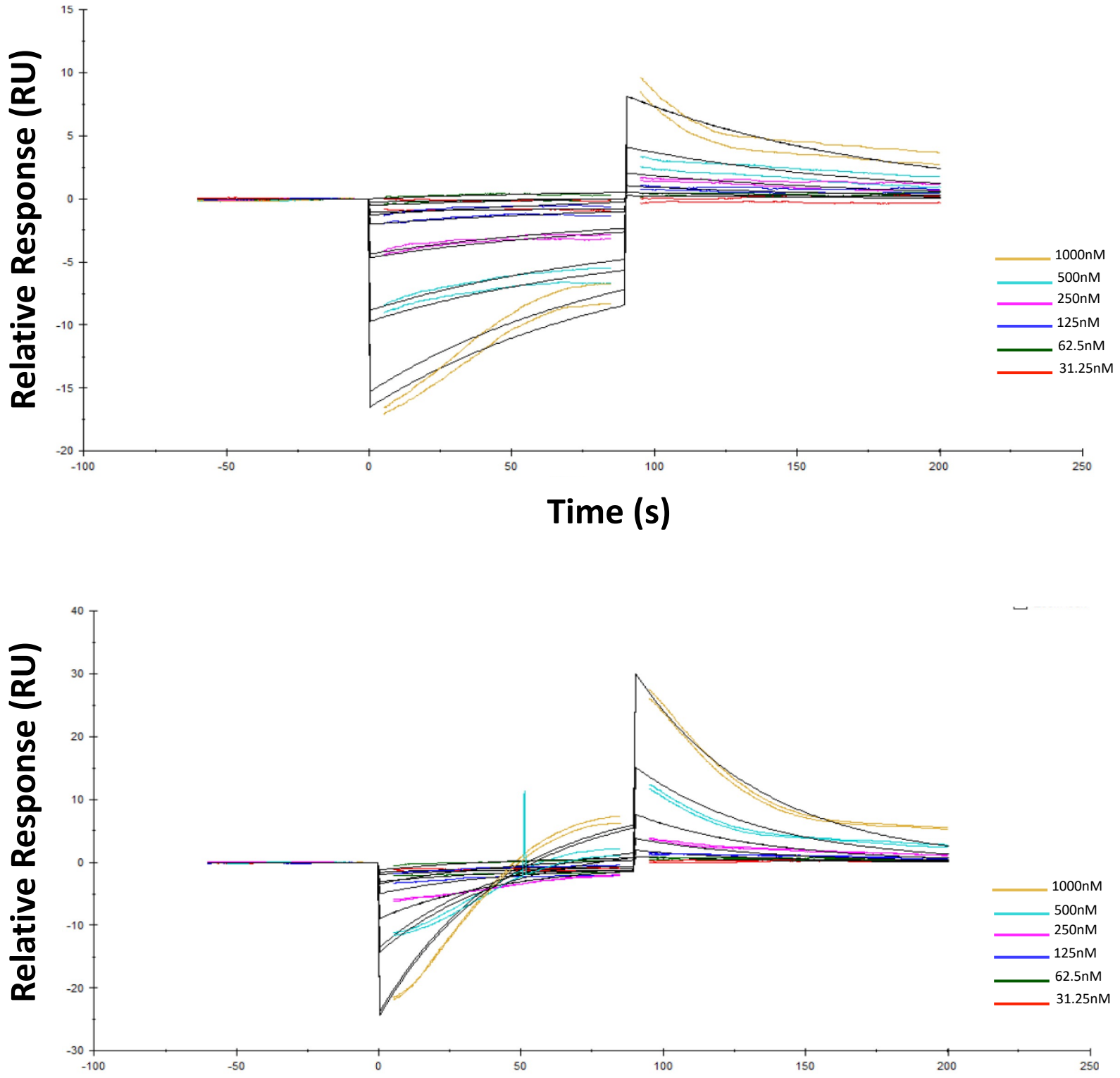


Figure S2: Mass photometry. molecular mass distribution of purified IgG Fc, $N1_{10\text{-}14}Fc$, and $N4_{10\text{-}14}Fc$ as measured by mass photometry. Blue shaded regions indicate detectable oligomerization states and their respective percentage of population.





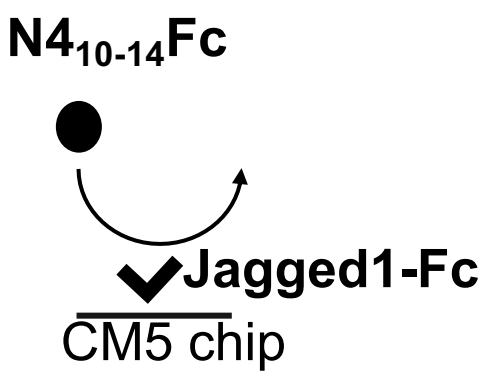
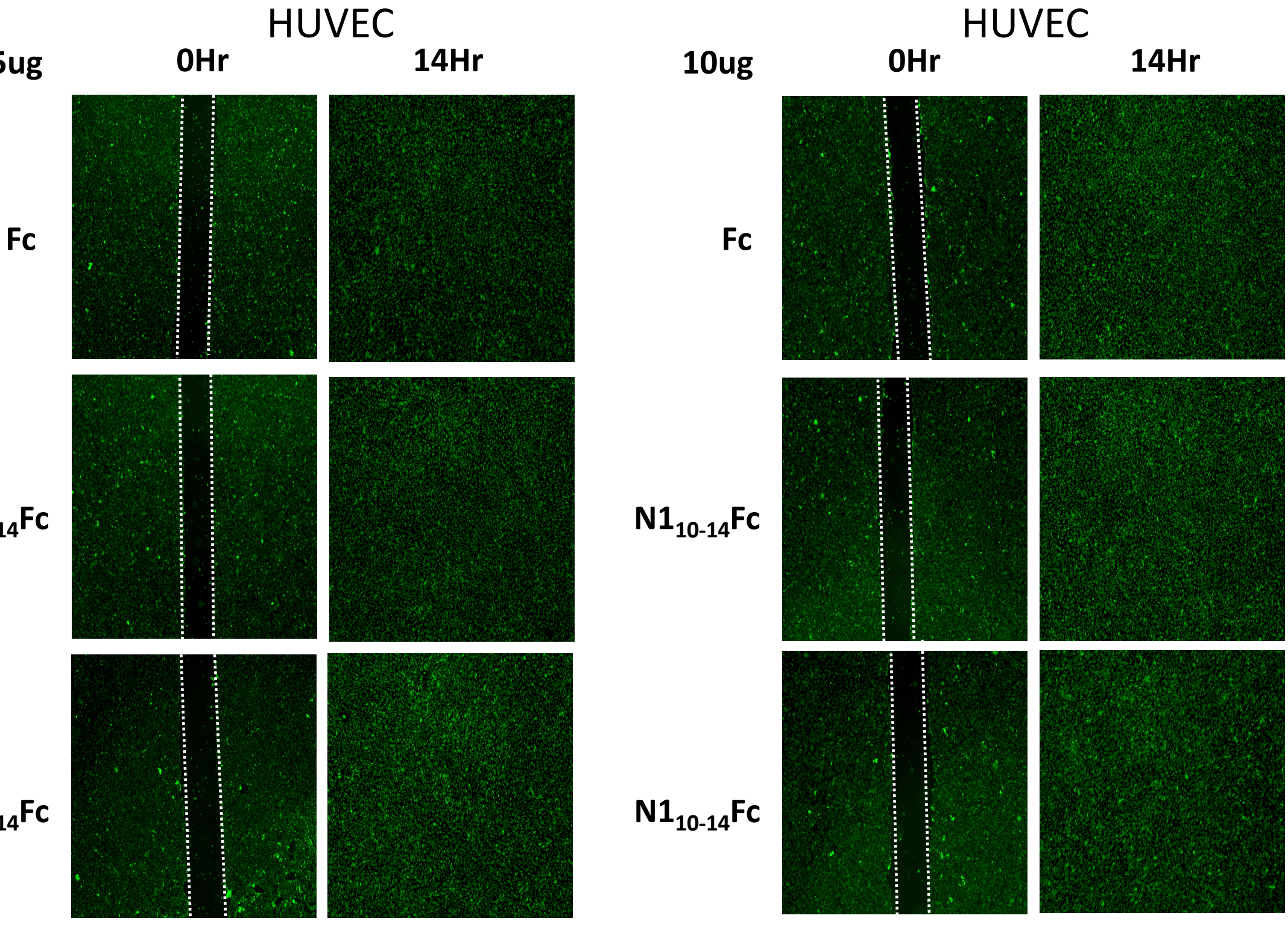


Figure S3: Binding of hJAG1-Fc to N1$_{10–14}$Fc and N4$_{10–14}$Fc. SPR measurements show binding affinity of N1$_{10-14}$Fc and N4$_{10-14}$Fc to hJAG1. Recombinant Fc-tagged hJAG1 was immobilized on the sensor chip using amine coupling and multi-cycle kinetic experiments were performed using increasing concentrations of either N1$_{10-14}$Fc or N4$_{10-14}$Fc. Binding was insufficient to allow for reliable calculation of Kd values.

A



B

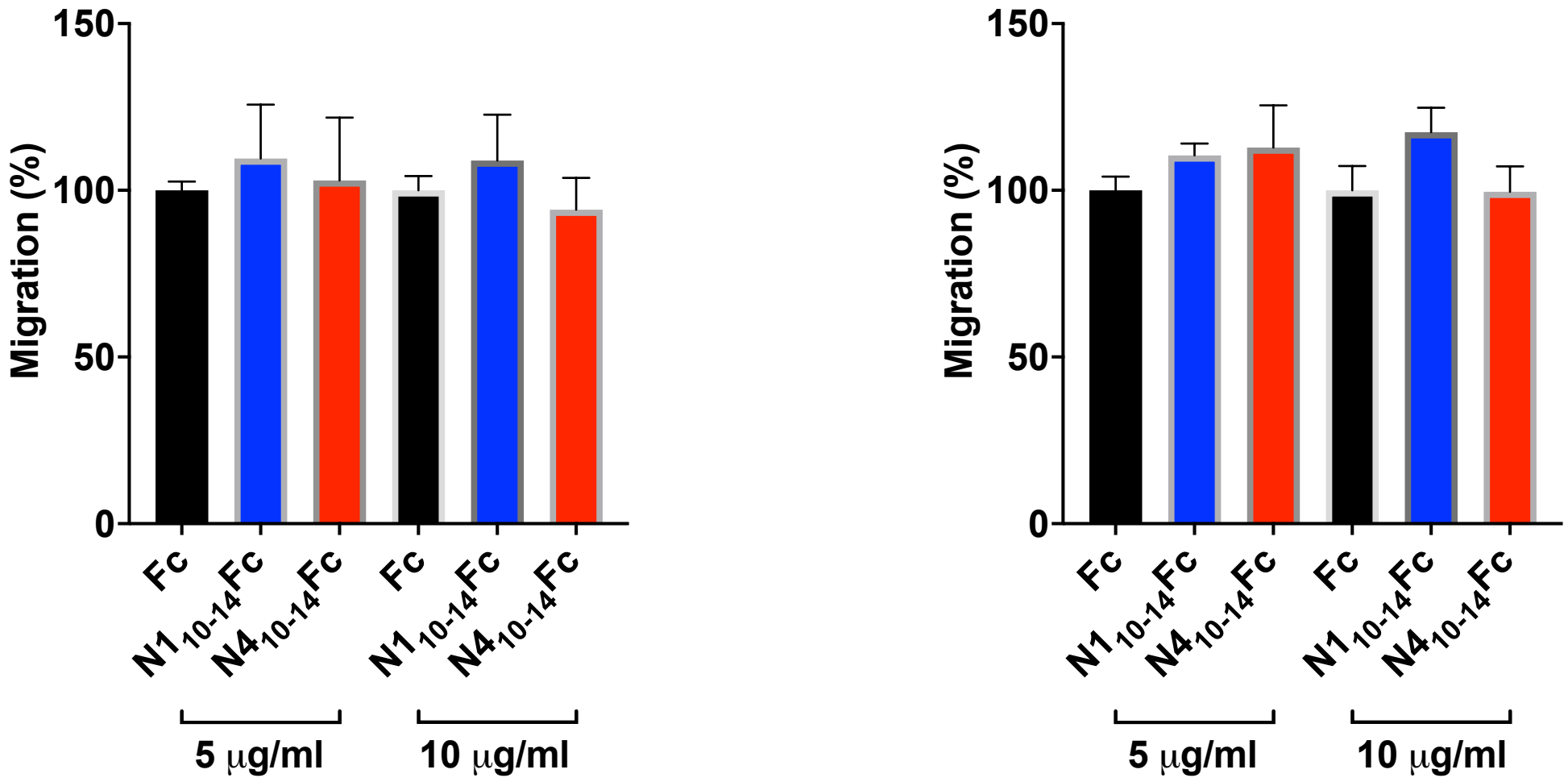


Figure S4: Wound healing assay of endothelial cells treated with Notch peptibodies. (A) HUVEC treated with cell-tracker CMFDA dye were seeded in 24-well plates and "scratch-wounded" using a 200 µl pipette tip. After wounding, cells were treated with different concentrations (5 or 10 ug/ul) of either IgG Fc, $N1_{10–14}Fc$ or $N4_{10–14}Fc$. After 14 hours, microscopy was used to image cell migration into the scratch region. (B) Quantitation of identical experiments performed with mouse endothelial cells (mLMVEC) and assessed at 14 and 20 hours.

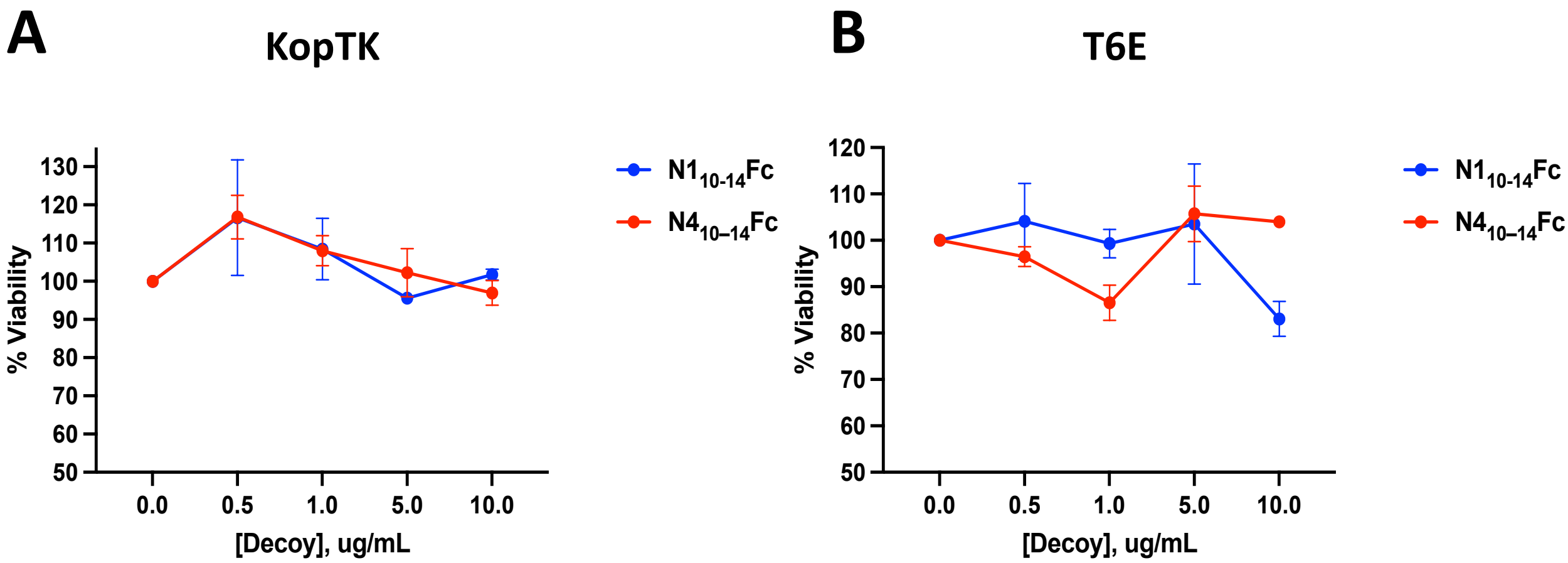


Figure S5: Viability assay of human and mouse T cell acute lymphoblastic leukemia (T-ALL) treated with Notch peptibodies. (A) human KopTK and (B) mouse T6E T-ALL cells were seeded at 8x10^3 cells per well in a 96-well plate in RPMI media and treated with the indicated concentration of IgG Fc, N$1_{10–14}$Fc or N$4_{10–14}$Fc for 72 hours. Viability in peptibody treated cells was assessed as the percent viable cells relative to the IgG Fc treated control group. Error bars indicate standard error of mean. For T-ALL experiments, n=3 for panel A and n=2 for panel B, no differences were significant.

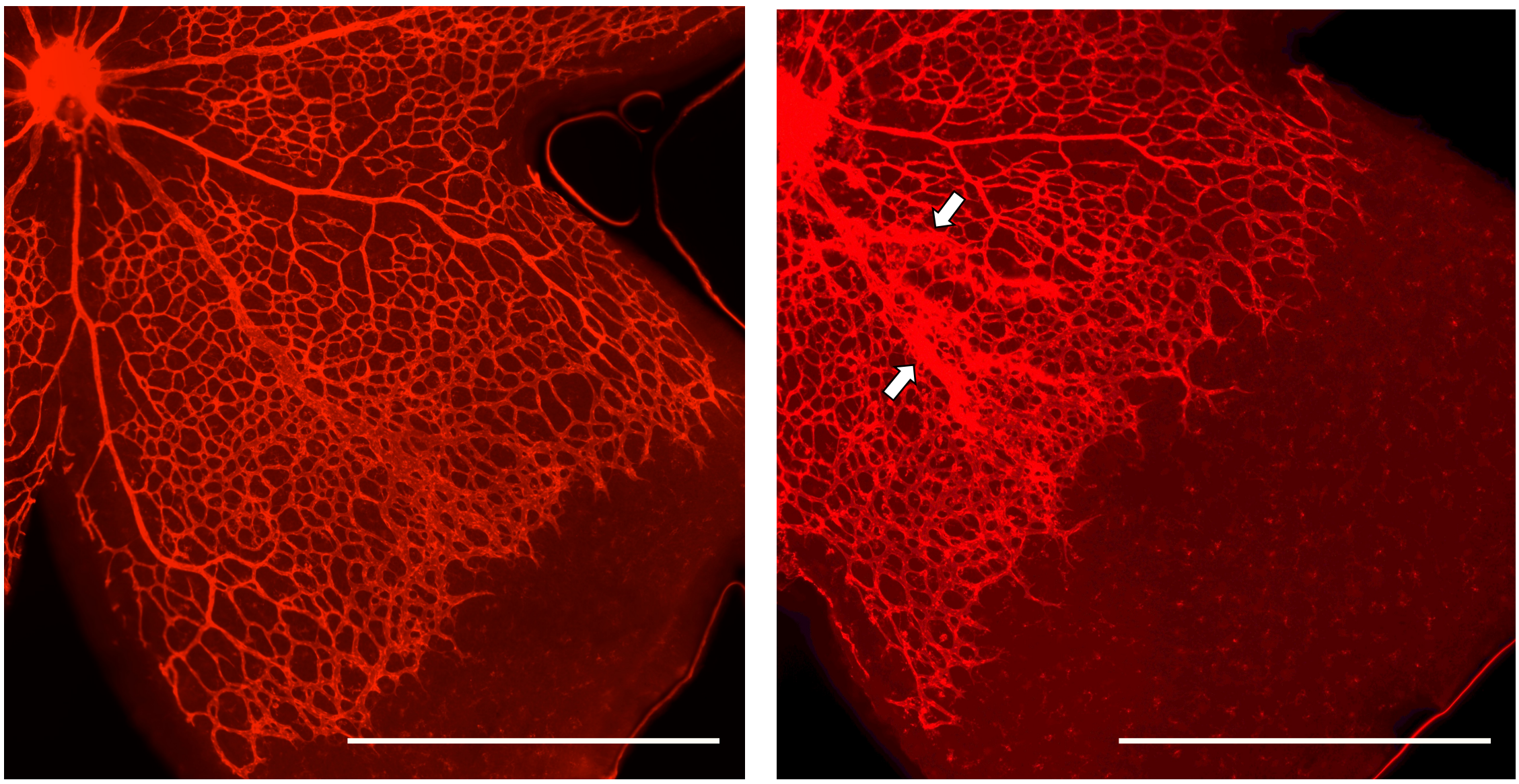


Figure S6: N1$_{10–14}$Fc induces abnormal enlargement of veins *in vivo*. C57BL/6 mice were injected intragastrically with 12.5 mg/kg of recombinant N1$_{10-14}$Fc decoy or IgG Fc for three days postnatally (P1-P3). (A) Representative images showing post-natal day 5 (P5) retinal vasculature stained with Isolectin B4 (red). (A) Arrowed region highlights enlarged veins. Scale bars: 1000 μm.

TABLE 1: QPCR PRIMERS

Species	Gene	Forward Sequence (5’-3’)	Reverse Sequence (5’-3’)
Human	ACTB	CGAGGCCCAGAGCAAGAGAG	CTCGTAGATGGGCACAGTGTG
Human	HEY1	ATCTGCTAAGCTAGAAAAAGCCG	GTGCGCGTCAAAGTAACCT
Human	HEY2	GCCCGCCCTTGTCAGTATC	CCAGGGTCGGTAAGGTTTATTG
Human	HES1	CCTGTCATCCCCGTCTACAC	CACATGGAGTCCGCCGTAA
Human	NRARP	TCAACGTGAACTCGTTCGGG	ACTTCGCCTTGGTGATGAGAT
Human	RND1	CTATCCAGAGACCTATGTGCC	CGGACATTATCGTAGTAGGGAG
Human	CCL2	AGGTGACTGGGGCATTGAT	GCCTCCAGCATGAAAGTCTC
Human	CCL8	TTCTGTGCCTGCTGCTCATG	TTGGATGTTGGTGATTCTTGTGTAG
Human	NOTCH1	GTCATCTCCGACTTCATCTACC	CGGAATCAGAGCGTGAGTAG
Human	NOTCH4	AGATAAATGGGGGAAAACTGCG	CCTGGGCATCTTTATCGGCT

Table S1: Primers used in RT-qPCR. Table of sequences of qPCR primers.