

SUPPLEMENTAL MATERIALS

Supplemental Figure 1: eA1 G-H loop mutants exhibiting similar activity to eA1-WT. Western blot of EphA2 immunoreactivity in U-251 MG cells treated with CM from: eA1-F108A, eA1-K118A, eA1-K121A for 24 hr. Equivalent dosing was verified by Western blotting; eA1-Fc (1 mg/ml) and eA1-WT CM were used as positive controls.

Supplemental Figure 2: Other than alanine substitutions at position 115 of eA1 do not retain an ability to down-regulate EphA2. Western blot of EphA2 immunoreactivity in U-251 MG cells treated with CM from T115R, T115D, and T115G for 24 hr.

Supplemental Figure 3: Binding of eA1-WT measured by SPR. SPR-determined profiles for eA1 monomer and dimer binding to EphA2. Maximum binding signals (RU) vs. concentration were obtained in a BIOcore T100 instrument by delivering each protein (dimer, squares; monomer, circles) to a EphA2 covalently coupled to a CM5 biosensor. Fitting the data to a single-site saturable binding model by nonlinear regression (SigmaPlot 11, SYSTAT Software, Santa Fe, CA) yielded $B_{max} = 229 \pm 7$ RU, $K_d = 89 \pm 21$ nM for eA1-Fc (solid line); monomer, $B_{max} = 163 \pm 26$ RU, $K_d = 580 \pm 240$ nM (dashed line).

Supplemental Figure 4: GBM cells rounding caused by eA1-WT and recombinant eA1-mutants. A) 1.0 μ g/ml and **B)** 0.1 μ g/ml concentrations were used. Phase-contrast microscopy after 30 min treatment of U-251 MG GBM cells. The pictures were taken at 20x magnification.

Supplemental Table 1: eA1 G-H loop alanine substitution mutants and primers used for site-directed mutagenesis.

Figure s2

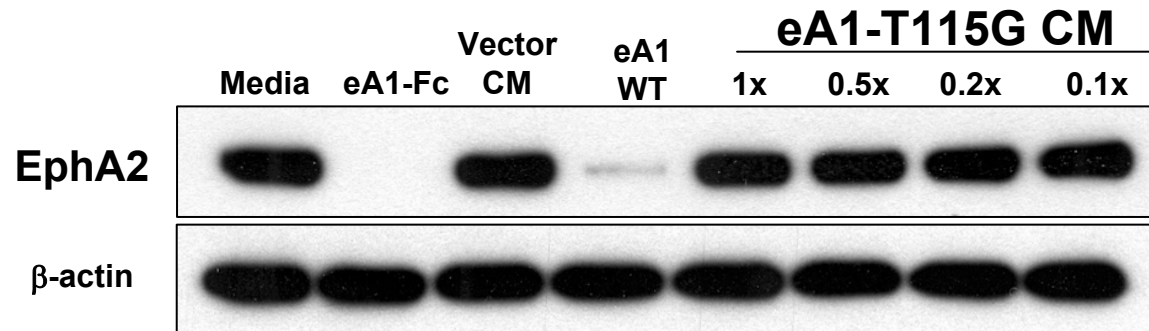
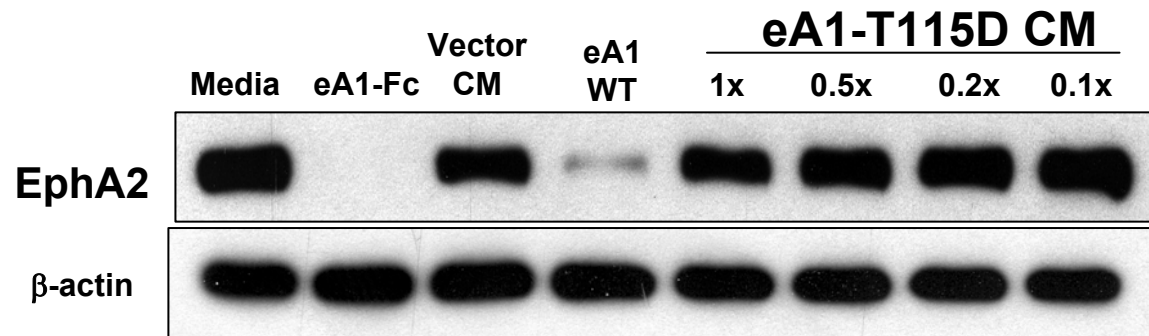
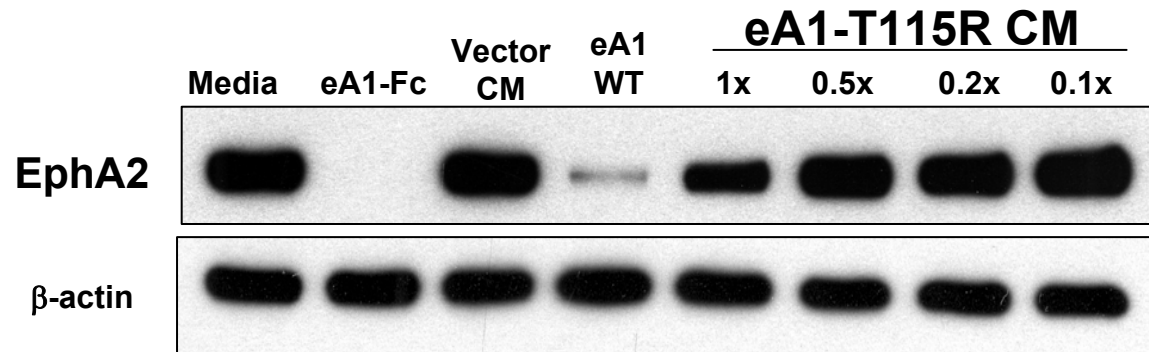


Figure s3

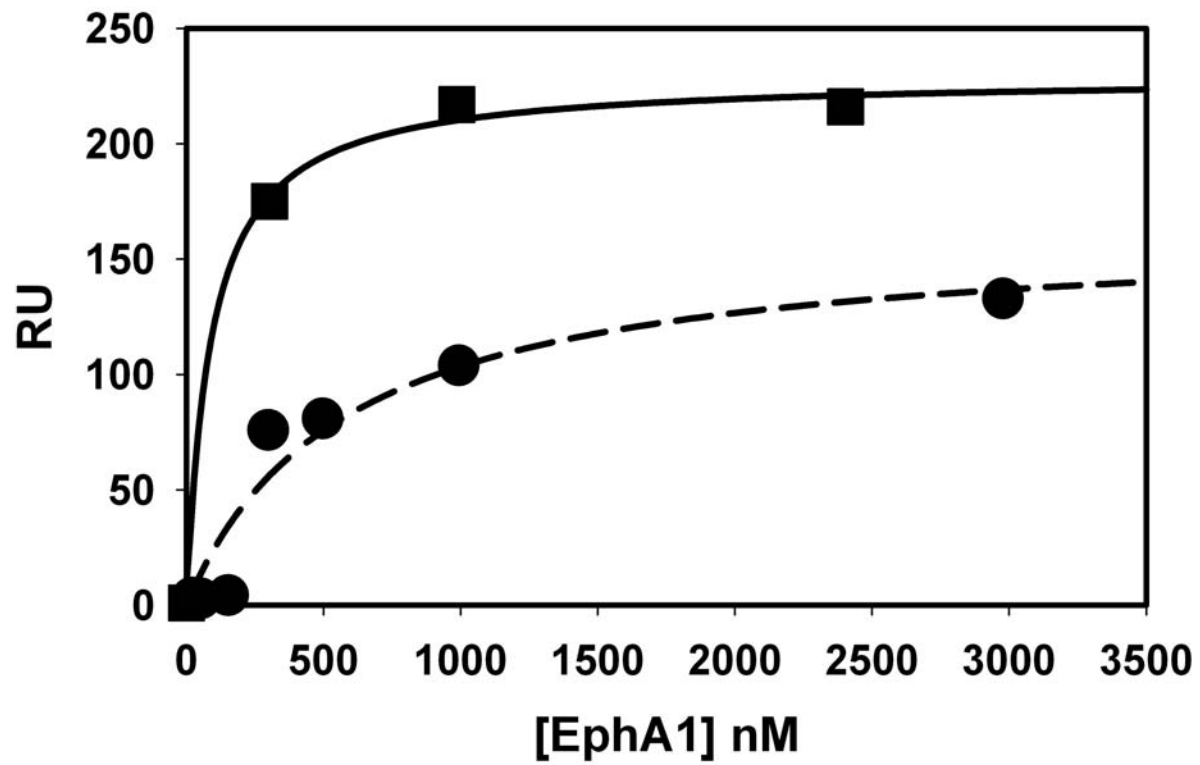


Figure s4A

eA1 (1.0 $\mu\text{g/ml}$)

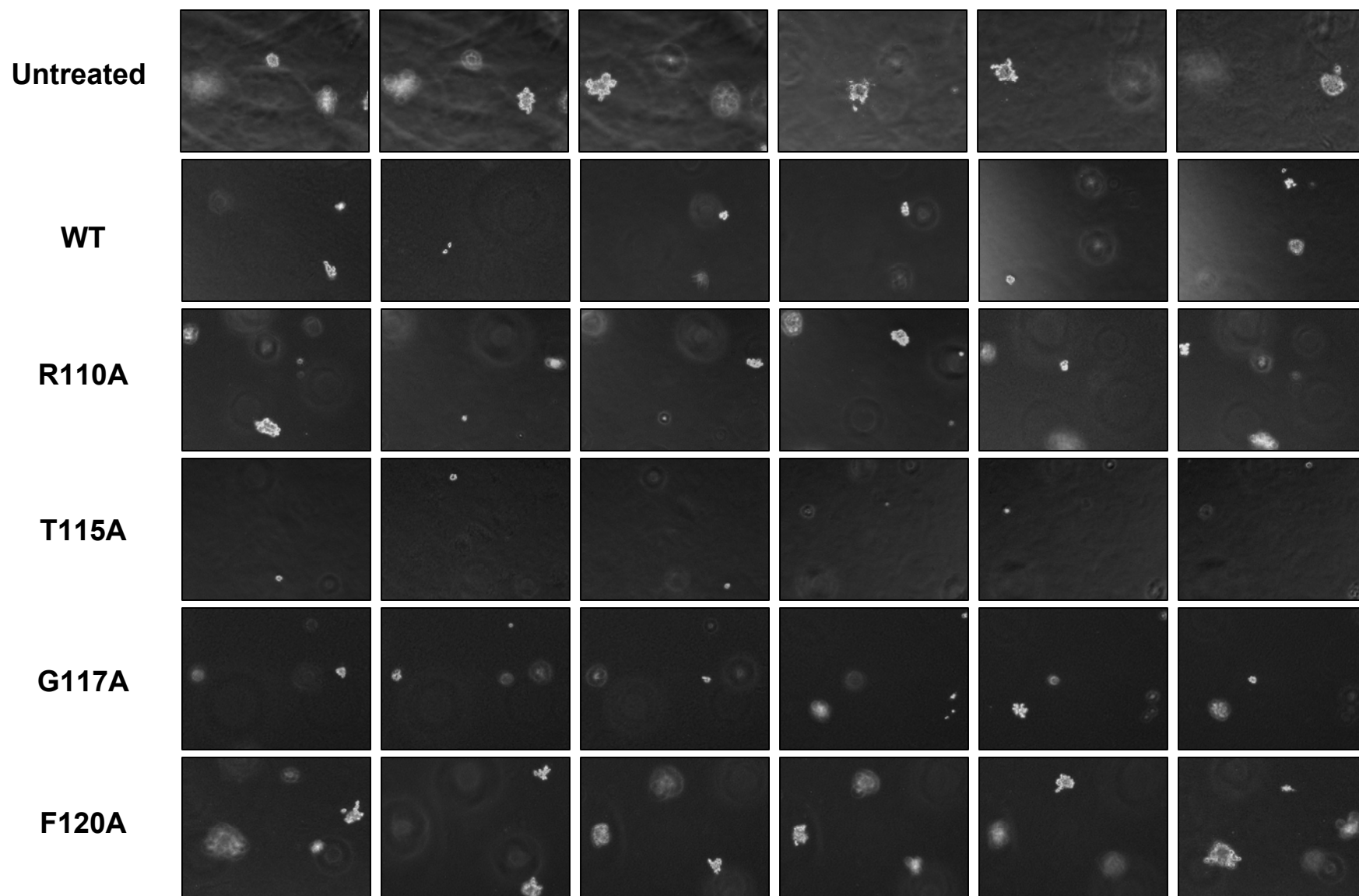
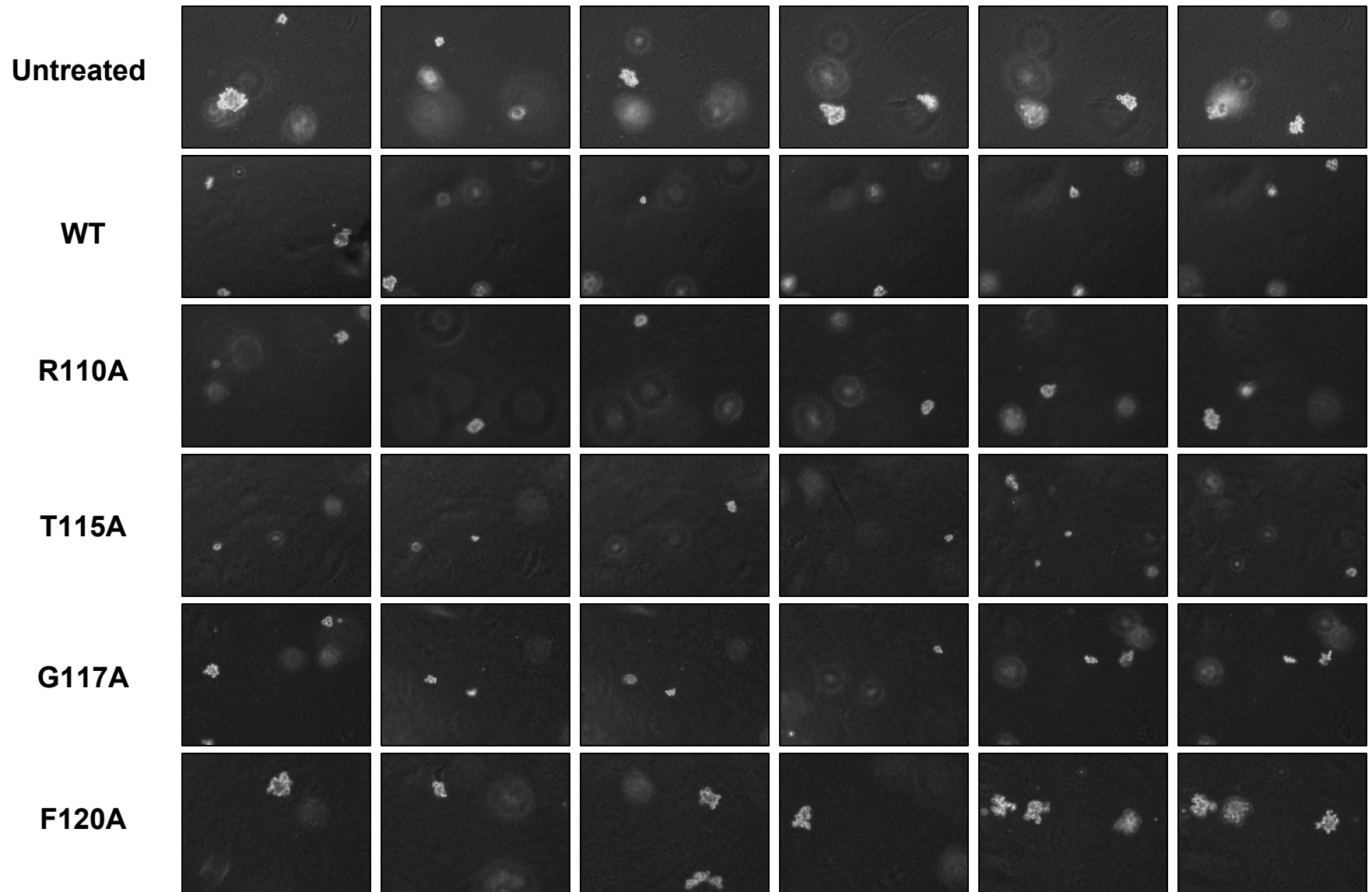


Figure s4B

eA1 (0.1 $\mu\text{g/ml}$)



Supplemental Table 1: eA1 G-H loop alanine substitution mutants and primers used for site-directed mutagenesis.

Mutant	Primer
F108A	5'-GAAGCTGTCTGAGAAGGCCCAACGCTTCACACCTT-3'
Q109A	5'-GCTGTCTGAGAAGTTCGCGCGCTTCACACCTTTCAC-3'
R110A	5'-CTGTCTGAGAAGTTCAGGCCTTCACACCTTTCACCCCTG-3'
F111A	5'-GAAGTTCAGCGCGCCACACCTTTCACCCTG-3'
T112A	5'-GTCTGAGAAGTTCAGAGGTTTCGCACCTTTCACCCTGGG-3'
P113A	5'-GTTCCAGCGCTTCACAGCTTTCACCCTGGGCAAG-3'
F114A	5'-GTTCCAGCGCTTCACACCTGCCACATTGGGCAAGGAGTTCAAAG-3'
T115A	5'-GCGCTTCACACCTTTCGCATTGGGCAAGGAGTTCAAAG3'
L116A	5'-GCGCTTCACACCTTTCACCGCAGGCAAGGAGTTCAAAGAAGG-3'
G117A	5'- TTCACCCTGGGCAAGGCGTTCAAAGAAGGACACAG-3'
K118A	5'-CACACCTTTCACCCTGGGCGCGGAGTTCAAAGAAGGACAC-3'
E119A	5'-TTCACACCTTTCACCCTGGGCAAGGCGTTCAAAGAAGGACACA-3'
F120A	5'- CACACCTTTCACCCTGGGCAAGGAGGCCAAAGAAGGACAC-3'
K121A	5'-CACCTGGGCAAAGAGTTCGCAGAAGGACACAGC-3'
E122A	5'-CACCTGGGCAAAGAGTTCAAAGCAGGACACAGC-3'
G123A	5'-CCCTGGGCAAAGAGTTCAAAGAAGCACACAGCTAC-3'