Supplementary Fig.2 Konno *et al.*

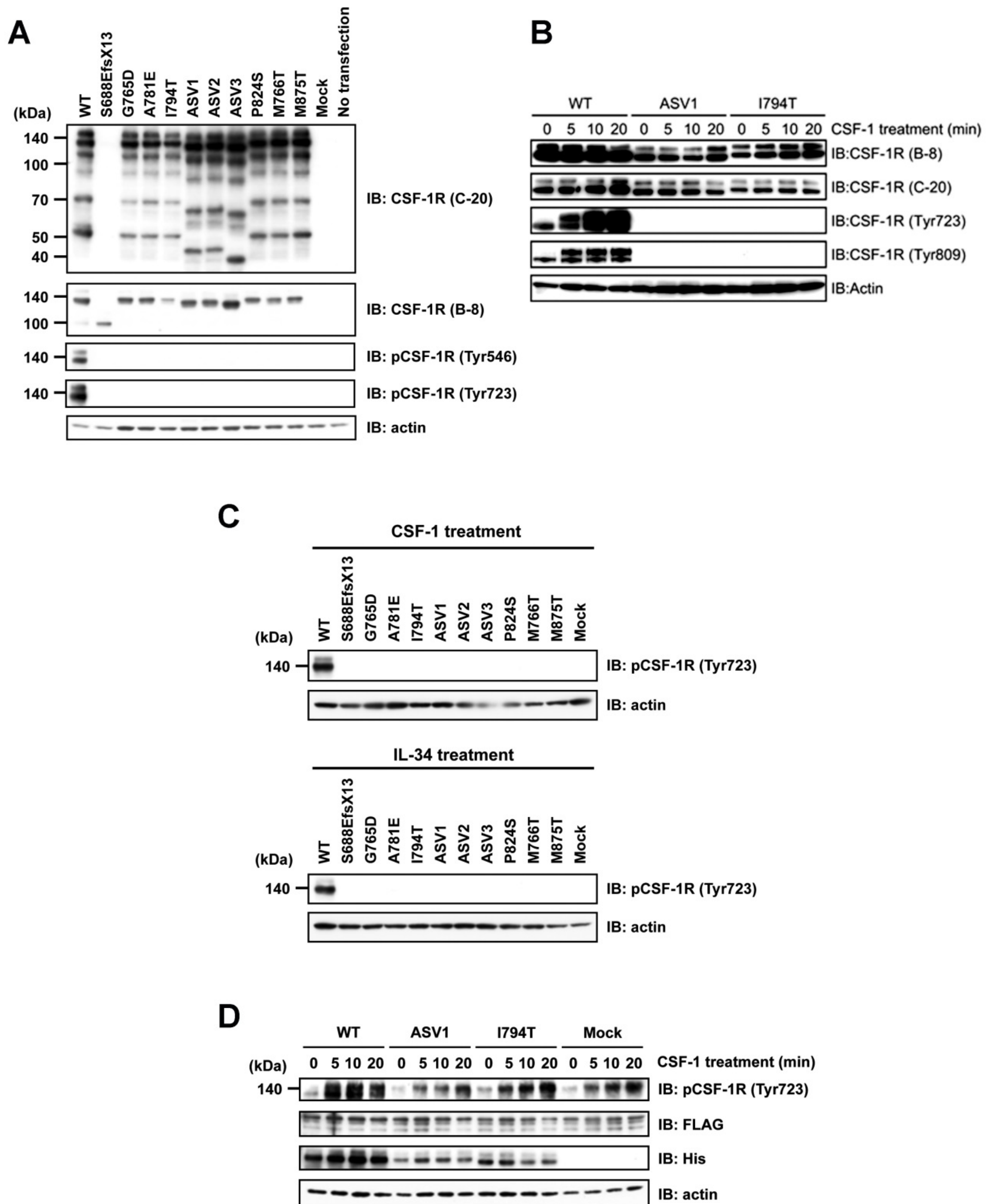

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Figure e-2. Impaired CSF-1R signaling in cells expressing mutant CSF-1R
(A) Expression of total CSF-1R protein in HEK293T cells transiently transfected with cDNA encoding wild-type and mutant CSF-1Rs (S688EfsX13, G765D, A781E, I794T, aberrant splicing variants (ASVs 1-3), P824S, M766T, and M875T). Detergent-extracted lysates were subjected to SDS-PAGE, followed by immunoblot analysis using anti-CSF-1R (C-20 and B-8) and anti-phospho-CSF-1R (Tyr 546 and 723) antibodies. Western blot analysis using anti-CSF-1R antibodies revealed comparable expression levels between wild-type and mutant CSF-1Rs. Note that mutant CSF-1R of S688EfsX13 lacking the C-terminal portion of CSF-1R showed no band reactive to the C-terminal antibody (C-20), but the N-terminal antibody recognized truncated CSF-1R migrating at 100 kDa.
(B) Ligand-dependent autophosphorylation of CSF-1R. HEK293T cells transiently transfected with cDNA encoding wild-type or mutant CSF-1R (ASV1 and I794T) were stimulated with 25 ng/mL CSF-1. Detergent-extracted lysates were collected at the indicated time after CSF-1 stimulation, and subjected to SDS-PAGE. Immunoblot analysis was performed using anti-CSF-1R and anti-phosphorylated CSF-1R antibodies. Note that increased levels of phosphorylated CSF-1R were observed in cells expressing wild-type CSF-1R in a time-dependent manner after CSF-1 stimulation, whereas neither of the mutant CSF-1Rs underwent autophosphorylation of CSF-1R following CSF-1 stimulation.
(C) Autophosphorylation of CSF-1R by CSF-1 and IL-34 stimulation. HEK293T cells transiently transfected with cDNA encoding wild-type or mutant CSF-1Rs were stimulated with CSF-1 (upper panel) or IL-34 (lower panel). Detergent-extracted lysates were collected 10 min after stimulation of the ligand. Cells expressing wild-type CSF-1R underwent autophosphorylation by CSF-1 as well as IL-34 stimulation, whereas cells expressing mutant CSF-1R showed no autophosphorylation following stimulation of the ligand.
(D) Lack of dominant-negative effect of missense CSF-1R mutants. HEK293 cells stably expressing wild-type FLAG-tagged CSF-1R were further transfected with myc-His-tagged wild-type or mutant CSF-1R. FLAG-tagged CSF-1R-expressing cells transfected with wild-type myc-His-tagged CSF-1R showed enhanced CSF-1R autophosphorylation compared with the mock-transfected FLAG-tagged CSF-1R-expressing cells. Notably, expression of the mutant myc-His-tagged CSF-1Rs fails to suppress the level of CSF-1R autophosphorylation of the mock-transnsfected FLAG-tagged CSF-1R-expressing cells.