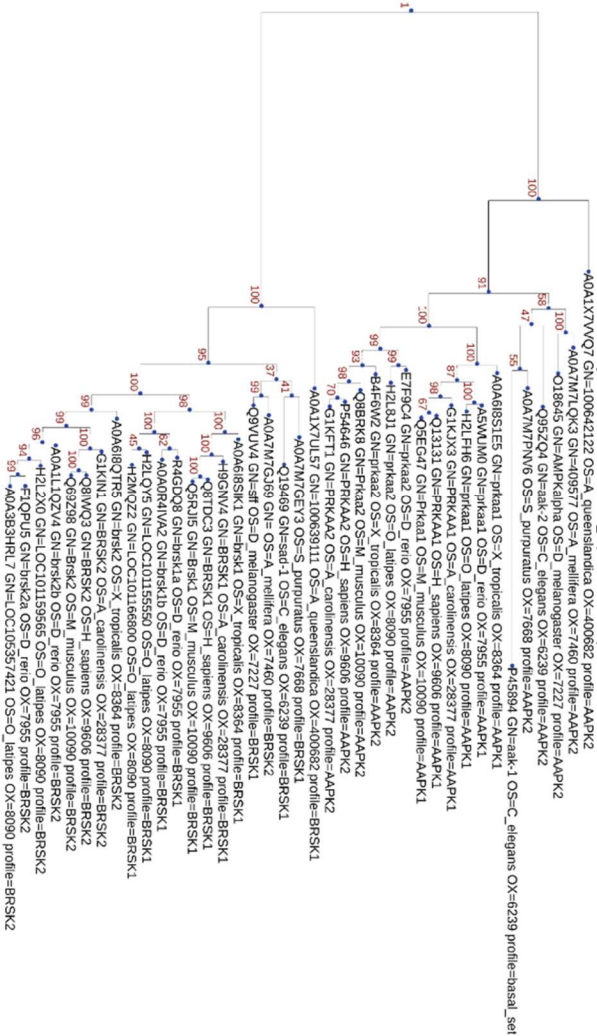
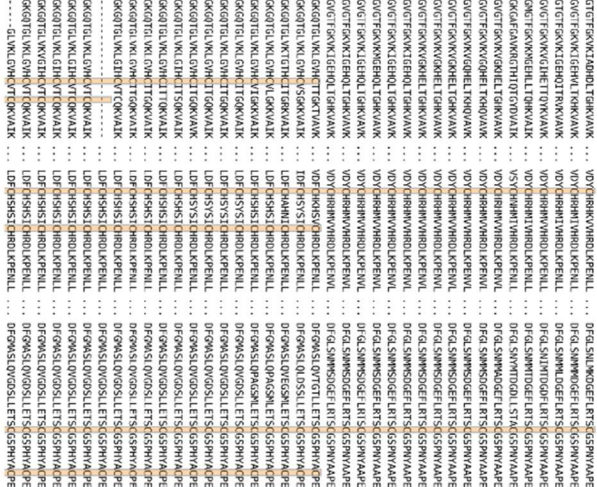


a



b2-83 Loop



HRD Motif

T-Loop

BRSK Cys

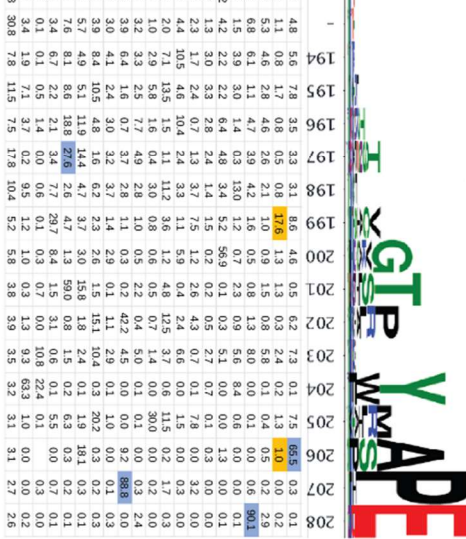
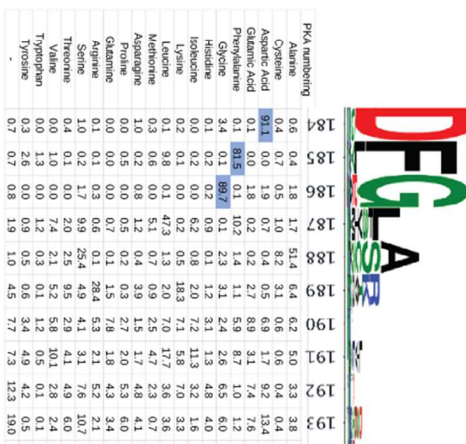
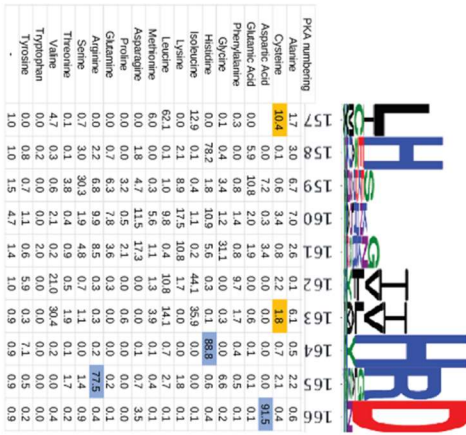
EPK Motif

1246

b

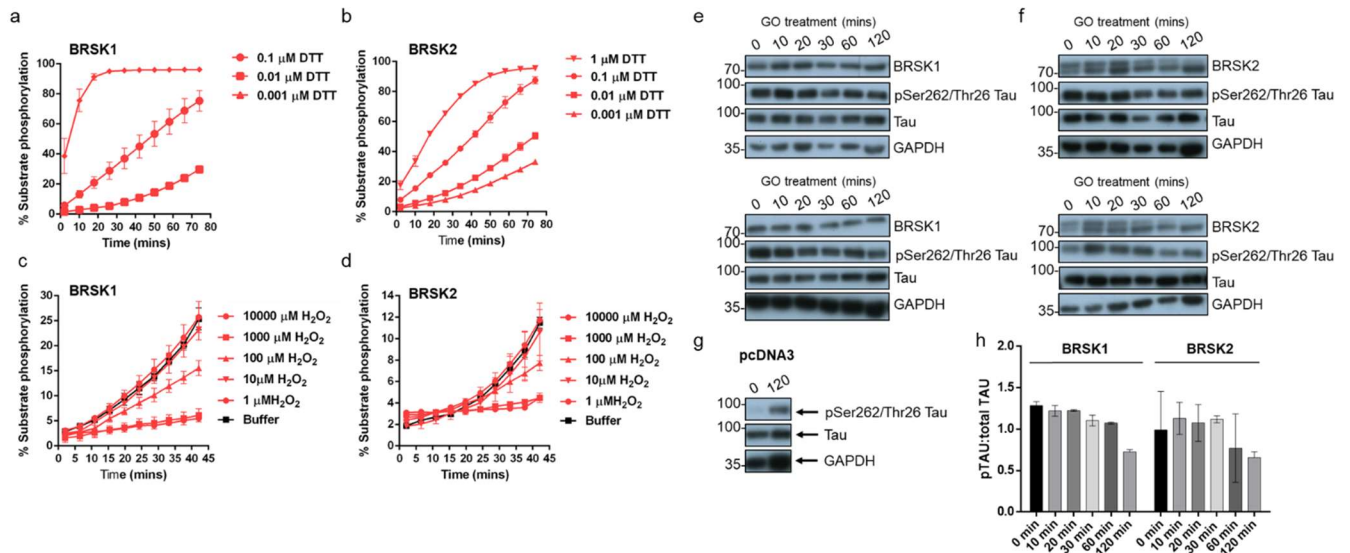
HRD Motif

T-Loop



1247

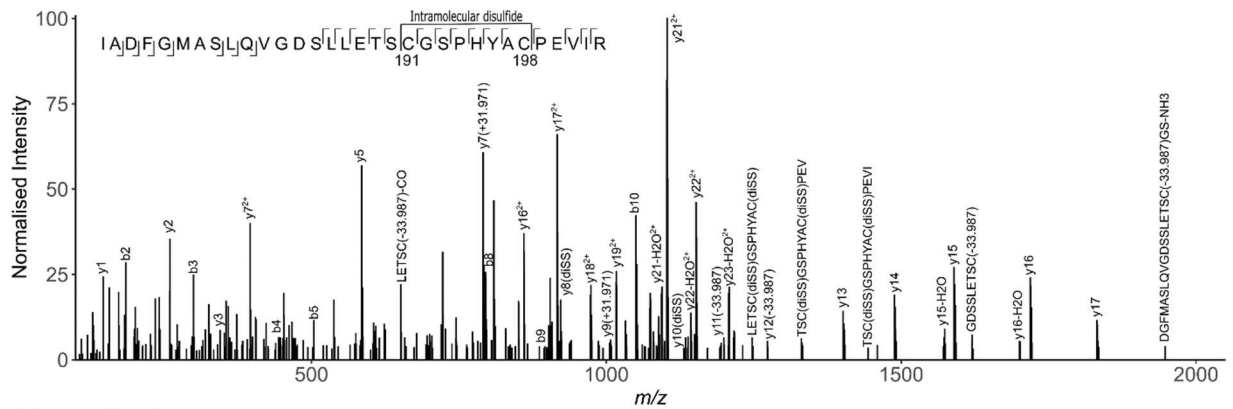
1248 Supplementary Figure 1: Phylogenetic analysis of BRSK and the ARK family. (a)
1249 Phylogenetic analysis of the ARK family reveals that the closest relative of BRSK
1250 kinases is AMPK. The number of cysteines in the kinase domain of BRSKs
1251 increases relative to AMPK. (b) Sequence alignment and relative amino acid
1252 composition of the activation segment of ePKs (top). Data is presented as HMM
1253 (hidden Markov models) Sequence Logos. Table (bottom) depicts the frequency
1254 of an amino acid at each position along the Catalytic and T-Loop. Key Cys
1255 residues are highlighted in orange; residues highly conserved in ePK canonical
1256 kinase motifs are highlighted in blue.
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1259



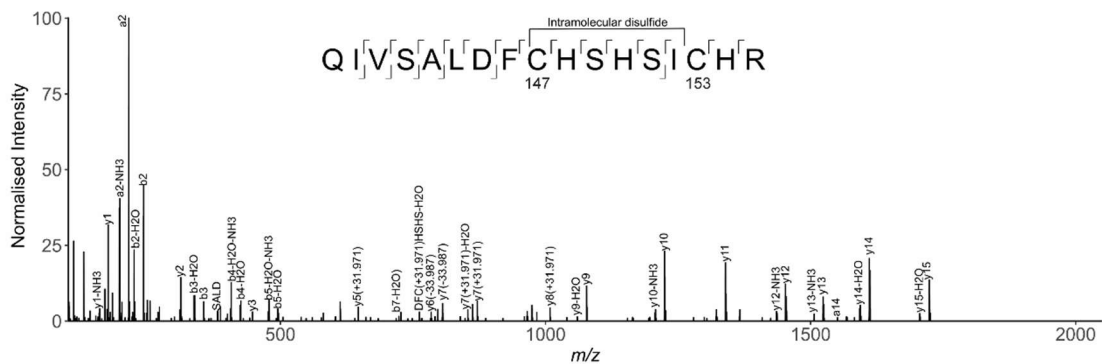
Supplementary Figure 2: Redox regulation of BRSK1 & 2. (a-d) Real-time phosphorylation of fluorescent AMARA peptide by full length BRSK1 and 2 (200 ng). BRSK proteins were incubated with buffer or the indicated concentrations of DTT or H₂O₂. Rates of BRSK activity were calculated as pmol per min phosphate incorporation and are presented in Fig 1. Data shown here is a subset of the conditions shown in Fig 1 (mean and SD from three repeats). (e-h) Time dependent loss of pTAU by incubation of HEK-293T cells with 2U/ml glucose oxidase (GO). Cells were transiently co-transfected with EGFP-Tau and either (e) BRSK1, (f) BRSK2 or (g) empty vector (pcDNA3). Data shown is WB analysis from 2 independent repeats. (h) pTau: Tau signals calculated with ImageJ. Data shown is mean and SD, calculated from (e) and (f).

1260

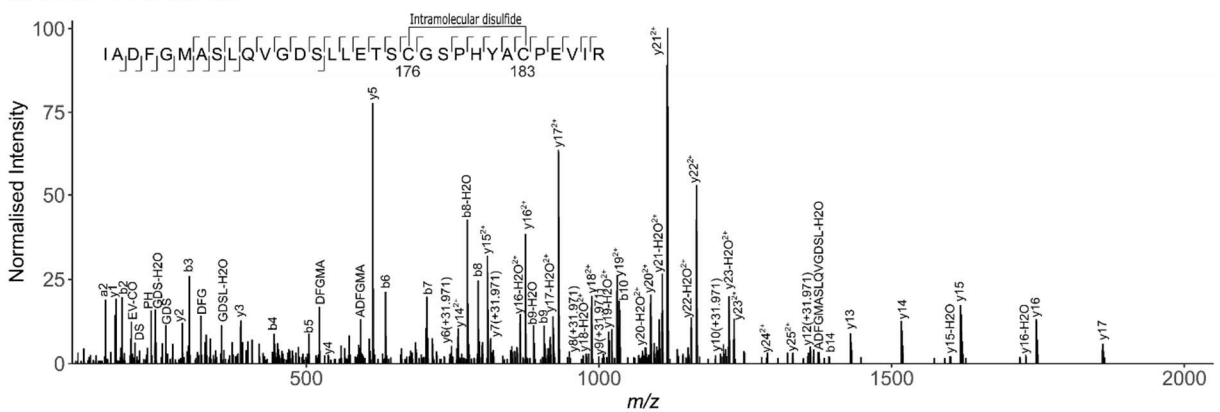
BRSK1 WT SAMPLE



BRSK1 WT SAMPLE

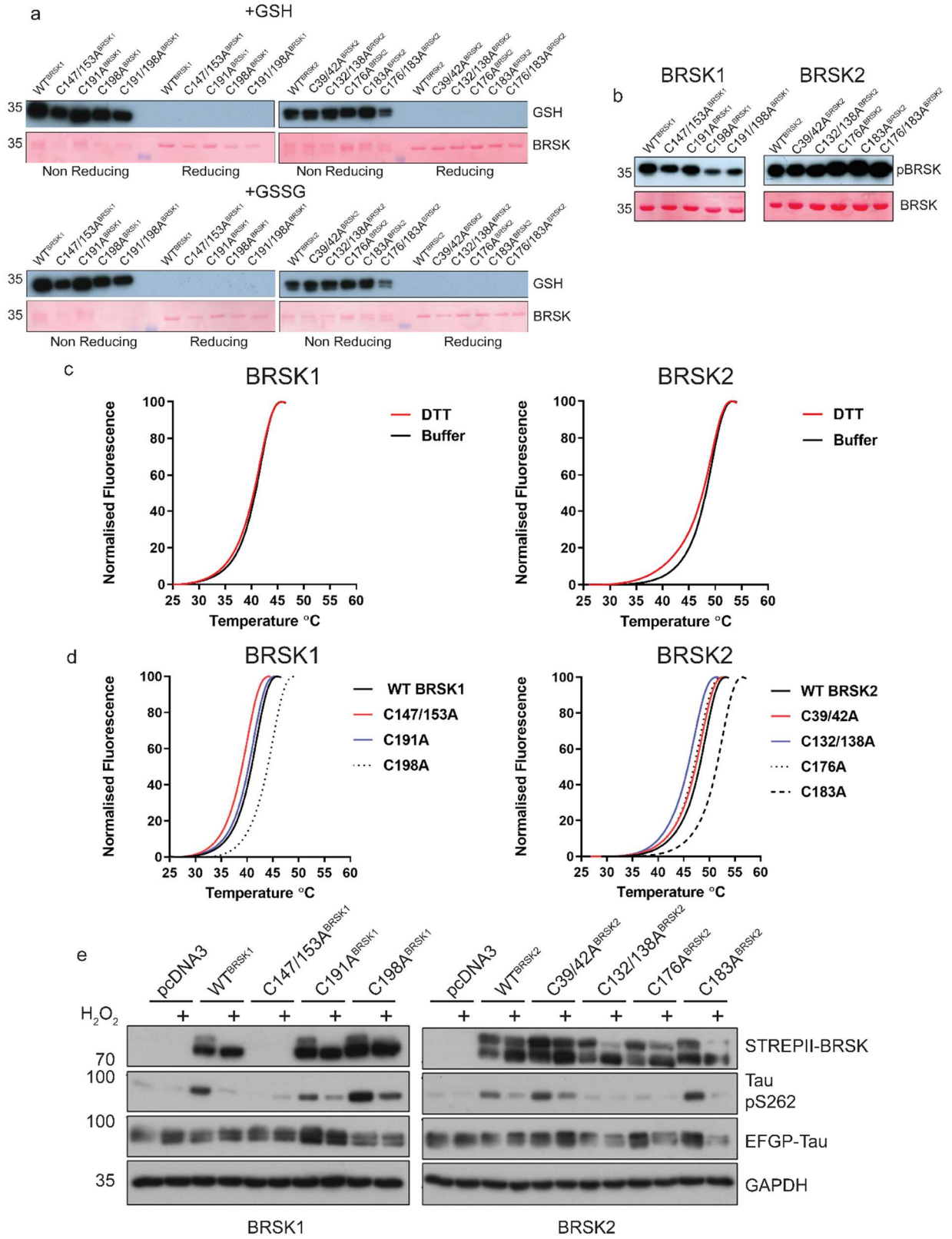


BRSK 2 WT SAMPLE

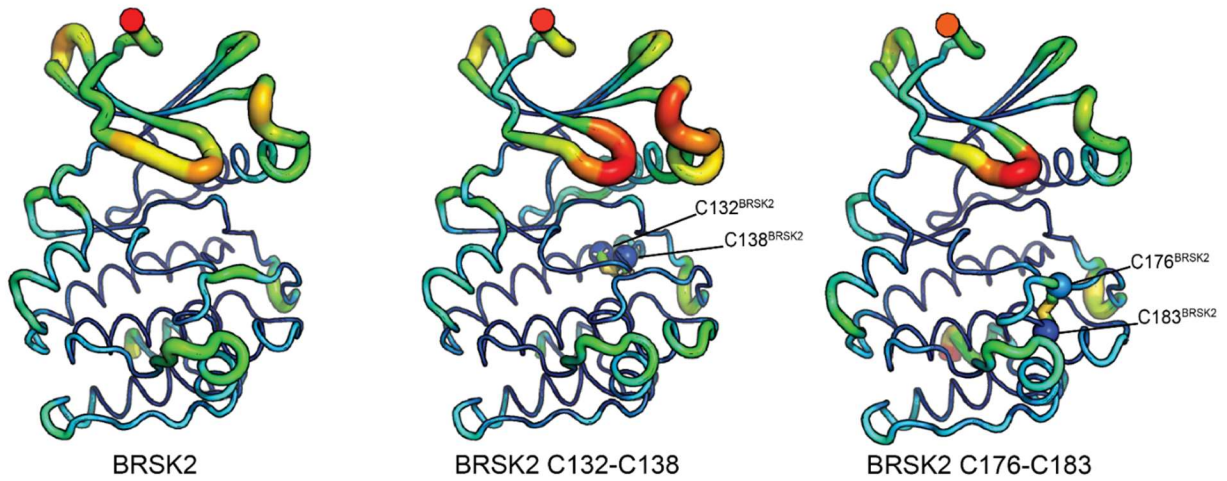


1261

1262 Supplementary Figure 3: LC-MS/MS Analysis of BRSK1/2 catalytic domains. LC-
 1263 MS/MS reveals intramolecular disulfide bonds in the kinase domains of BRSK1
 1264 and 2 purified from *E. coli*.



1266 Supplementary Figure 4: Biochemical analysis of BRSK Cys-to Ala mutants. (a)
1267 Immunoblot of in vitro glutathionylation of BRSK kinase domains. (b) Immunoblot
1268 showing LKB1-dependent phosphorylation of BRSK kinase domain proteins. (c)
1269 Thermal denaturation curves of BRSK catalytic domain proteins in the presence
1270 or absence of 10 mM DTT. (d) Thermal denaturation curves of BRSK catalytic
1271 domain cysteine to alanine mutants. (e) Representative immunoblot of EGFP-
1272 Tau co-expressed with full length, WT and Cys-to-Ala mutants of BRSK1 and
1273 BRSK2. Transiently transfected HEK-293T cells were treated with or without 10
1274 mM H₂O₂ for 10 mins.



1275 Supplementary Figure 5: Molecular Dynamics Simulations of intramolecular disulfide
1276 bonds. Simulations incorporating disulfide bonds identified in MS/MS
1277 experiments. RMSF was calculated based on three 100 ns GROMACS molecular
1278 dynamics simulations. Higher mobility is indicated by warmer colors and
1279 thickness of representation.
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1281
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