Hsp70\_w\_wt MA**K**AAAIGIDLGTTYSCVGVFQHG**K**VEIIANDQGNRTTPSYVAFTDTERLIGDAA**K**NQVA 60

Hsp70\_w\_k0 MA**K**AAAIGIDLGTTYSCVGVFQHG**K**VEIIANDQGNRTTPSYVAFTDTERLIGDAA**K**NQVA 60

Hsp70\_w\_k48r MA**K**AAAIGIDLGTTYSCVGVFQHG**K**VEIIANDQGNRTTPSYVAFTDTERLIGDAA**K**NQVA 60

Hsp70\_1.5 MA**K**AAAIGIDLGTTYSCVGVFQHG**K**VEIIANDQGNRTTPSYVAFTDTERLIGDAA**K**NQVA 60

Hsp70\_w\_wt LNPQNTVFDA**K**RLIGR**K**FGDPVVQSDM**K**HWPFQVINDGD**K**P**K**VQVSY**K**GET**K**AFYPEEIS 120

Hsp70\_w\_k0 LNPQNTVFDA**K**RLIGR**K**FGDPVVQSDM**K**HWPFQVINDGD**K**P**K**VQVSY**K**GET**K**AFYPEEIS 120

Hsp70\_w\_k48r LNPQNTVFDA**K**RLIGR**K**FGDPVVQSDM**K**HWPFQVINDGD**K**P**K**VQVSY**K**GET**K**AFYPEEIS 120

Hsp70\_1.5 LNPQNTVFDA**K**RLIGR**K**FGDPVVQSDM**K**HWPFQVINDGD**K**P**K**VQVSY**K**GET**K**AFYPEEIS 120

Hsp70\_w\_wt SMVLT**K**M**K**EIAEAYLGYPVTNAVITVPAYFNDSQRQAT**K**DAGVIAGLNVLRIINEPTAAA 180 Hsp70\_w\_k0 SMVLT**K**M**K**EIAEAYLGYPVTNAVITVPAYFNDSQRQAT**K**DAGVIAGLNVLRIINEPTAAA 180

Hsp70\_w\_k48r SMVLT**K**M**K**EIAEAYLGYPVTNAVITVPAYFNDSQRQAT**K**DAGVIAGLNVLRIINEPTAAA 180

Hsp70\_1.5 SMVLT**K**M**K**EIAEAYLGYPVTNAVITVPAYFNDSQRQAT**K**DAGVIAGLNVLRIINEPTAAA 180

Hsp70\_w\_wt IAYGLDRTG**K**GERNVLIFDLGGGTFDVSILTIDDGIFEV**K**ATAGDTHLGGEDFDNRLVNH 240

Hsp70\_w\_k0 IAYGLDRTG**K**GERNVLIFDLGGGTFDVSILTIDDGIFEV**K**ATAGDTHLGGEDFDNRLVNH 240

Hsp70\_w\_k48r IAYGLDRTG**K**GERNVLIFDLGGGTFDVSILTIDDGIFEV**K**ATAGDTHLGGEDFDNRLVNH 240

Hsp70\_1.5 IAYGLDRTG**K**GERNVLIFDLGGGTFDVSILTIDDGIFEV**K**ATAGDTHLGGEDFDNRLVNH 240

Hsp70\_w\_wt FVEEF**K**R**K**H**KK**DISQN**K**RAVRRLRTACERA**K**RTLSSSTQASLEIDSLFEGIDFYTSITRA 300 Hsp70\_w\_k0 FVEEF**K**R**K**H**KK**DISQN**K**RAVRRLRTACERA**K**RTLSSSTQASLEIDSLFEGIDFYTSITRA 300

Hsp70\_w\_k48r FVEEF**K**R**K**H**KK**DISQN**K**RAVRRLRTACERA**K**RTLSSSTQASLEIDSLFEGIDFYTSITRA 300

Hsp70\_1.5 FVEEF**K**R**K**H**KK**DISQN**K**RAVRRLRTACERA**K**RTLSSSTQASLEIDSLFEGIDFYTSITRA 300

Hsp70\_w\_wt RFEELCSDLFRSTLEPVE**K**ALRDA**K**LD**K**AQIHDLVLVGGSTRIP**K**VQ**K**LLQDFFNGRDLN 360 Hsp70\_w\_k0 RFEELCSDLFRSTLEPVE**K**ALRDA**K**LD**K**AQIHDLVLVGGSTRIP**K**VQ**K**LLQDFFNGRDLN 360

Hsp70\_w\_k48r RFEELCSDLFRSTLEPVE**K**ALRDA**K**LD**K**AQIHDLVLVGGSTRIP**K**VQ**K**LLQDFFNGRDLN 360

Hsp70\_1.5 RFEELCSDLFRSTLEPVE**K**ALRDA**K**LD**K**AQIHDLVLVGGSTRIP**K**VQ**K**LLQDFFNGRDLN 360

Hsp70\_w\_wt **K**SINPDEAVAYGAAVQAAILMGD**K**SENVQDLLLLDVAPLSLGLETAGGVMTALI**K**RNSTI 420 Hsp70\_w\_k0 **K**SINPDEAVAYGAAVQAAILMGD**K**SENVQDLLLLDVAPLSLGLETAGGVMTALI**K**RNSTI 420

Hsp70\_w\_k48r **K**SINPDEAVAYGAAVQAAILMGD**K**SENVQDLLLLDVAPLSLGLETAGGVMTALI**K**RNSTI 420

Hsp70\_1.5 **K**SINPDEAVAYGAAVQAAILMGD**K**SENVQDLLLLDVAPLSLGLETAGGVMTALI**K**RNSTI 420

Hsp70\_w\_wt PT**K**QTQIFTTYSDNQPGVLIQVYEGERAMT**K**DNNLLGRFELSGIPPAPRGVPQIEVTFDI 480 Hsp70\_w\_k0 PT**K**QTQIFTTYSDNQPGVLIQVYEGERAMT**K**DNNLLGRFELSGIPPAPRGVPQIEVTFDI 480

Hsp70\_w\_k48r PT**K**QTQIFTTYSDNQPGVLIQVYEGERAMT**K**DNNLLGRFELSGIPPAPRGVPQIEVTFDI 480

Hsp70\_1.5 PT**K**QTQIFTTYSDNQPGVLIQVYEGERAMT**K**DNNLLGRFELSGIPPAPRGVPQIEVTFDI 480

Hsp70\_w\_wt DANGILNVTATD**K**STG**K**AN**K**ITITND**K**GRLS**K**EEIERMVQEAE**K**Y**K**AEDEVQRERVSA**K**N 540

Hsp70\_w\_k0 DANGILNVTATD**K**STG**K**AN**K**ITITND**K**GRLS**K**EEIERMVQEAE**K**Y**K**AEDEVQRERVSA**K**N 540

Hsp70\_w\_k48r DANGILNVTATD**K**STG**K**AN**K**ITITND**K**GRLS**K**EEIERMVQEAE**K**Y**K**AEDEVQRERVSA**K**N 540

Hsp70\_1.5 DANGILNVTATD**K**STG**K**AN**K**ITITND**K**GRLS**K**EEIERMVQEAE**K**Y**K**AEDEVQRERVSA**K**N 540

Hsp70\_w\_wt ALESYAFNM**K**SAVEDEGL**K**G**K**ISEAD**KKK**VLD**K**CQEVISWLDANTLAE**K**DEFEH**K**R**K**ELE 600 Hsp70\_w\_k0 ALESYAFNM**K**SAVEDEGL**K**G**K**ISEAD**KKK**VLD**K**CQEVISWLDANTLAE**K**DEFEH**K**R**K**ELE 600

Hsp70\_w\_k48r ALESYAFNM**K**SAVEDEGL**K**G**K**ISEAD**KKK**VLD**K**CQEVISWLDANTLAE**K**DEFEH**K**R**K**ELE 600

Hsp70\_1.5 ALESYAFNM**K**SAVEDEGL**K**G**K**ISEAD**KKK**VLD**K**CQEVISWLDANTLAE**K**DEFEH**K**R**K**ELE 600

Hsp70\_w\_wt QVCNPIISGLYQGAGG-PG--PGGF--GAQGP**K**GGSGSGPTIEEVD 641

Hsp70\_w\_k0 QVCNPIISGLYQGAGG-PG--PGGF--GAQGP**K**GGSGSGPTIEEVD 641

Hsp70\_w\_k48r QVCNPIISGLYQGAGG-PG--PGGF--GAQGP**K**GGSGSGPTIEEVD 641

Hsp70\_1.5 QVCNPIISGLYQGAGG-PG--PGGF--GAQGP**K**GGSGSGPTIEEVD 641

Figure S5. Details of the LC-MS/MS analysis of Hsc70-Ub when ubiquitinated by Ube2W and different forms of Ub. As in the previous figure, the sequence is annotated with observed regions in red, all lysines in bold, and ubiquitinated lysines in yellow.