Supporting Information

Hubert et al. 10.1073/pnas.0904877106

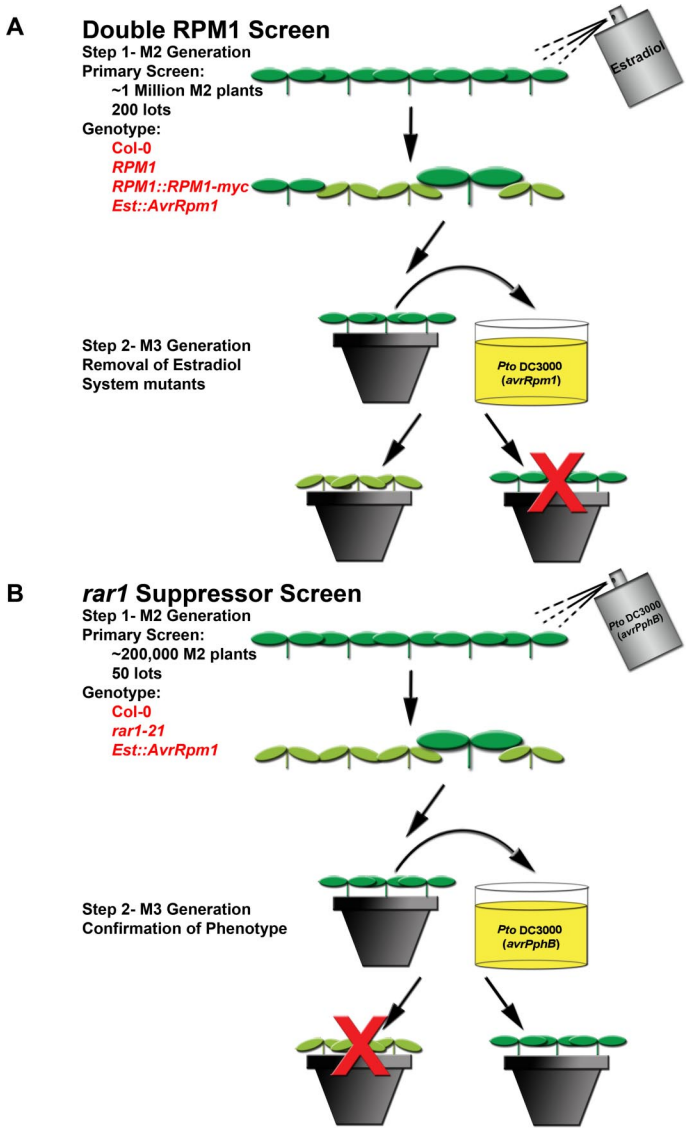

Fig. S1. Two genetic screens to identify genes involved in plant disease resistance. These flow charts depict the process conducted in both genetic screens. (*A*) The Double RPM1 Screen began with wild-type Columbia-0 plants expressing *RPM1*. An estradiol-inducible version of the bacterial gene *AvrRpm1* was introduced along with an *RPM1* transgene carrying a myc-epitope-tagged version of RPM1 under the control of its native promoter (1, 2). This line was mutagenized, and ≈100 M_1 plants were allowed to self in each of 200 separate pools or lots. *AvrRpm1* expression in the resulting M_2 plants was induced with estradiol. Seed was collected from nonresponsive plants. These M_3 plants were then tested for resistance to *Pto* DC3000(*avrRpm1*). This step allowed the identification and removal of plants with mutations in the estradiol-inducible expression system. (*B*) The *rar1* suppressor screen was begun by mutagenizing *rar1–21* mutant seed (2) carrying the same estradiol-inducible version of *AvrRpm1* as in *A*. The resulting M_1 plants were allowed to self in 50 separate lots. M_2 plants were sprayed with *Pto* DC3000(*avrPphB*). Disease-resistant plants were allowed to self, and resulting M_3 plants were retested by dip inoculation in separately in both *Pto* DC3000(*avrPphB*) and (*avrRpm1*) to confirm the disease-resistant phenotype.

1. Boyes DC, Nam J, Dangl JL (1998) The *Arabidopsis thaliana RPM1* disease resistance gene product is a peripheral plasma membrane protein that is degraded coincident with the hypersensitive response. *Proc Natl Acad Sci USA* 95:15849–15854.
2. Tornero P, et al. (2002) *RAR1* and *NDR1* contribute quantitatively to disease resistance in *Arabidopsis* and their relative contributions are dependent on the *R* gene assayed. *Plant Cell* 14:1005–1015.

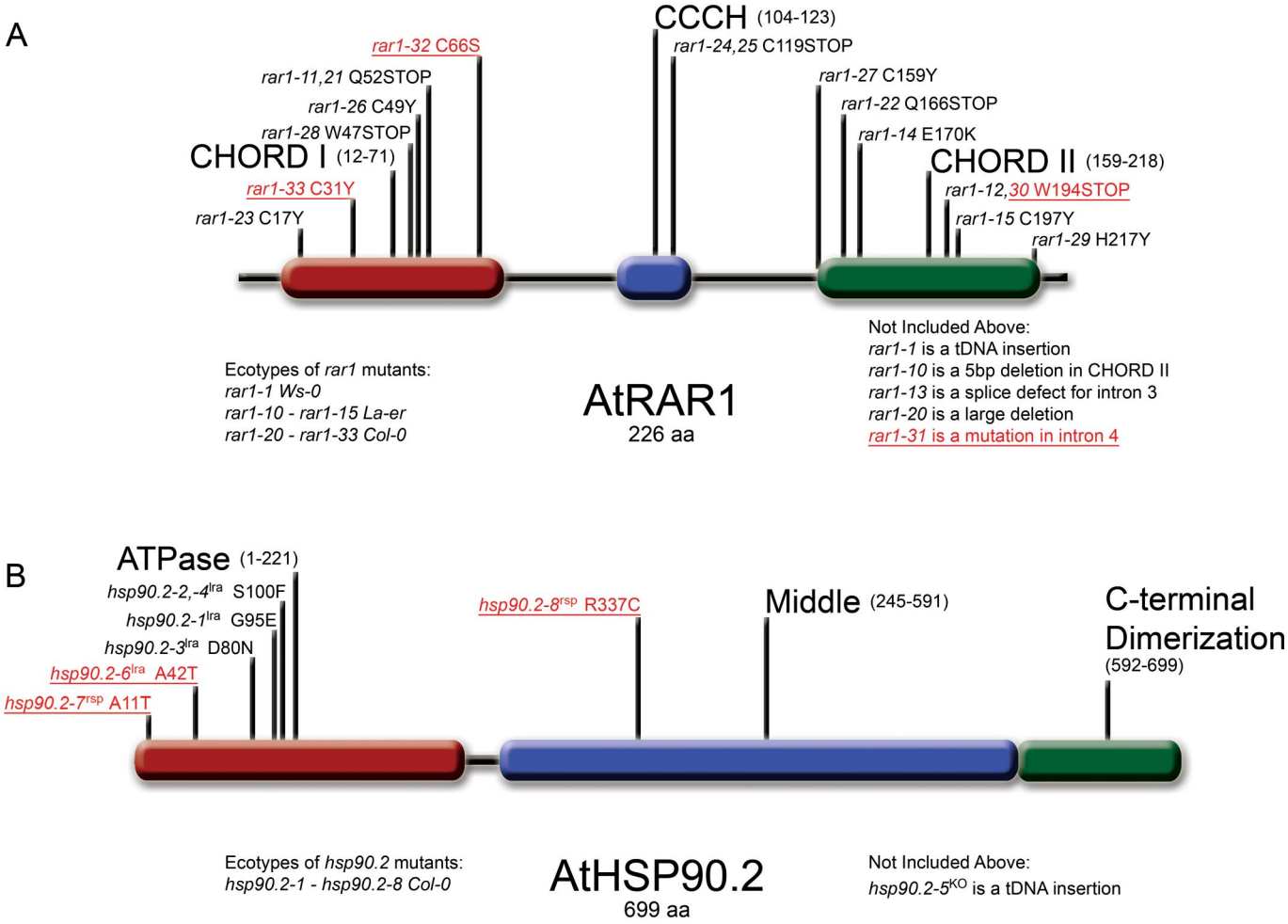

Fig. S2. Schematic representations of *Arabidopsis* RAR1 and HSP90.2 showing the location of all known mutant alleles. Alleles introduced in this article are underlined and in red. (*A*) Identified mutations in RAR1. The CHORD-I domain is shown in red, the CCCH region is shown in blue, and the CHORD-II domain is shown in green. The allele designation and associated amino acid change are shown in relation to its linear position. The ecotypes in which the mutants were identified are shown below. Noncoding mutations are described below the linear molecule. (*B*) Identified mutations in HSP90.2. The ATPase domain is shown in red, the middle domain is shown in blue, and the C-terminal dimerization domain is shown in green. The phenotype of the respective mutation is indicated after the allele designation and associated amino acid change, *lra* for loss of recognition of avrRpm1 and *rsp* for rar1 suppressor.

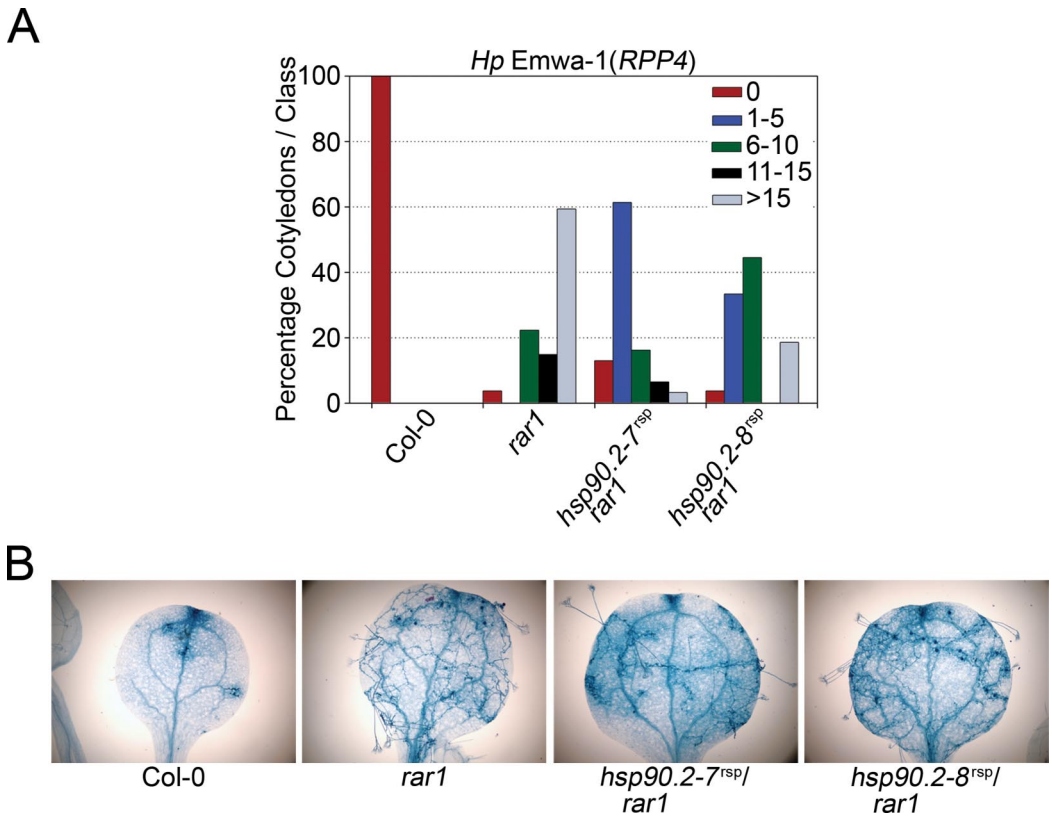

Fig. S3. *hsp90.2*^{rsp} alleles suppress the *rar1* effect on *RPP4*, a TIR-NB-LRR, in response to an oomycete pathogen, *Hpa*, isolate Emwa1. (*A*) The *RAR1*-dependent TIR-NB-LRR protein RPP4 conditions recognition of the *Hpa* isolate Emwa-1 in wild-type Col-0 plants. Consequently, there is no oomycete sporulation in these plants. RAR1 is required for RPP4-mediated recognition. Consequently, *rar1* mutants display a high level of *Hpa* reproduction. *hsp90.2*^{rsp} *rar1* double mutants both display more sporangiophores than wild-type plants but less than a *rar1* single mutant, suggesting partial suppression of *rar1* for RPP4 function. Colored bars refer to the number of sporangiophores counted per cotyledon. Minimum of 20 cotyledons per genotype was used. (*B*) Trypan blue staining of dead plant cells and oomycete hyphal structures is shown. Dead xylem cells in the vascular bundle can be seen in all genotypes. While areas of death representing a HR can be seen in Col-0, fine hair-like hyphae can be seen in, and reproductive sporangiophores are shown radiating from, the *rar1* cotyledon. The *hsp90.2*^{rsp} *rar1* double mutants support intermediate levels of *Hpa* growth and the trailing necrosis associated with partial NB-LRR function.

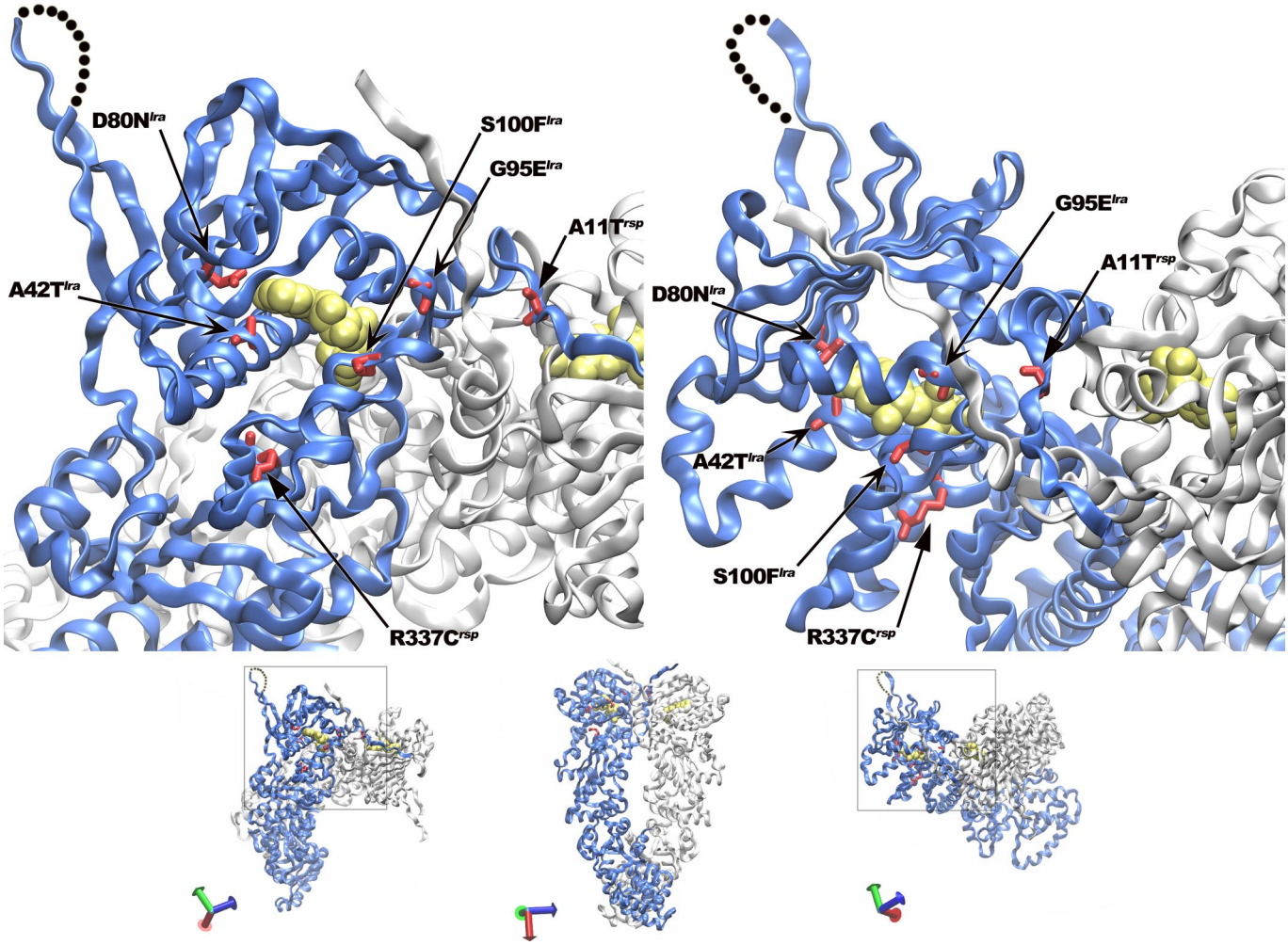

Fig. S4. All identified *AtHSP90.2* mutations in *Arabidopsis* lie within or near the ATPase domain. Ribbon structure close-up of the crystal structure of the yeast HSP90 dimer (Protein Data Bank ID code 2CG9) showing the locations of the residues mutated in AtHSP90.2 in red. Individual HSP90 monomers are shown in light gray and blue. The lra or rsp designation of each respective mutant is shown after the residue. ATP is shown in pale yellow. A view of the full dimer is shown underneath with the area of the close-up represented in a box. Axes are shown for orientation. Residue numbering relates to position in AtHSP90.2, inferred by alignment with yeast HSP90.

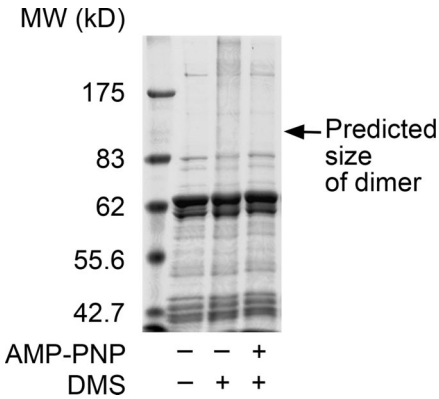

Fig. S5. The AtHSP90.2 ATPase domain is insufficient for dimer formation. A C-terminal truncation of HSP90 is unable to dimerize in a chemical cross-linking experiment. The protein was tested without nucleotide or with 10 mM of the nonhydrolysable ATP analog, AMP-PNP. The experiment shown was conducted at 0.25 mg/mL of HSP90 ΔC and 15 molar equivalents of DMS; similar results were obtained with 30 molar equivalents and the cross-linkers DSS and DMP.

Table S1. Previously-identified HSP90 mutations

Mutation position in reference to AtHSP90.2	Mutation position in reference to ScHSP90	Mutation position in original organism	Species	Mutant name	Protein	Phenotype	Source/ref.
Single amino acid changes							
E5R	E4R	E6R	*Triticum aestivum*		TaHSP90	Reduced binding to the CS domain of SGT1 and AtRAR1	1
A11T	A10T	A11T	*Arabidopsis thaliana*	A*thsp90.2–7*	AtHSP90.2	Restoration of NB-LRR function and accumulation in a rar1 mutant; normal ATPase activity; normal dimerization; normal RAR1 interaction; decreased SGT1 interaction	This study
T23I	T22I	T22I	*Saccharomyces cerevisiae*		ScHSP82	Impairs AhR signaling; temperature sensitivity; reduced GR activity; osmosensitive; increased ATPase activity; reduced interaction with cdc37; reduced accumulation of GR; enhanced AMP-PNP binding; enhanced N-terminal dimerization	2–7
T23I	T22I	T22I	*Saccharomyces cerevisiae*		ScHSC82	Temperature sensitive growth defect; reduced GR and v-Src activities; enhanced ATPase activity; reduced GR accumulation; reduced interactions with client protein Sti1 and Sba1	8
T23F	T22F	T22F	*Saccharomyces cerevisiae*		ScHSC82	Increases ATP hydrolysis	9
F24A	V23A	V23A	*Saccharomyces cerevisiae*		ScHSC82	4.8-fold reduction of ATP hydrolysis (compared with WT)	9
Y25A	Y24A	Y24A	*Saccharomyces cerevisiae*		ScHSC82	4.8-fold reduction of ATP hydrolysis	9
E292K	E301K	E292K	*Caenorhabditis elegans*	*daf-21(p673)*	CeHSP90	Defects in specific chemosensory responses; reduced fertility	10
E34A	E33A	E33A	*Saccharomyces cerevisiae*		ScHSP82	Decreased ATP hydrolysis	11, 12
E34A	E33A	E33A	*Saccharomyces cerevisiae*		ScHSC82	Abolishes ATP hydrolysis	9
E34A	E33A	E46A	*Gallus gallus*		HSP90α	Loses the ability to assist HSP70, HSP40 and HOP in the refolding protein; abolishes ATP hydrolysis	13
S36L	S37L	S38L	*Drosophila melanogaster*	*E(sev)3A e1D*	HSP83	Lethality; reduced Raf kinase activity; reduced binding to Raf	14, 15
N38A	N37A	N50A	*Gallus gallus*		HSP90α	Abolishes nucleotide binding and interacting with p23	13
A42V	A41V	A41V	*Saccharomyces cerevisiae*		ScHSP82	Impairs AhR signaling; temperature sensitivity; reduced GR activity; reduced accumulation of GR; enhanced AMP-PNP binding; reduced ATPase acitivity	3, 4, 6
A42T	A41T	A42T	*Arabidopsis thaliana*	A*thsp90.2–6*	AtHSP90.2	Loss of RPM1 function and accumulation; fully penetrant phenotype; loss of ATPase activity; loss of dimerization; loss of RAR1 interaction; loss of SGT1 interaction	This study
R47C	R46C	R48C	*Drosophila melanogaster*	*13F3*	HSP83	Reduced Raf kinase activity	15
D80N	D79N	D79N	*Saccharomyces cerevisiae*		ScHSP82	Growth retardation; decreased binding to ATP, ADP and p23	11, 12
D80N	D79N	D79N	*Saccharomyces cerevisiae*		ScHSC82	Reduced interactions with client protein Sba1 and Cpr6	8
D80N	D79N	D92A	*Gallus gallus*		HSP90α	Abolishes nucleotide binding and interacting with p23	13
D80N	D79N	D80N	*Arabidopsis thaliana*	A*thsp90.2–3*	AtHSP90.2	Loss of RPM1 function and accumulation; fully penetrant phenotype; loss of ATPase activity; loss of dimerization; loss of RAR1 interaction; loss of SGT1 interaction	16; this study
G82S	G81S	G81S	*Saccharomyces cerevisiae*		ScHSP82	Impairs AhR signaling; temperature sensitivity; reduced GR activity; osmosensitive	3, 4, 7

Mutation position in reference to AtHSP90.2	Mutation position in reference to ScHSP90	Mutation position in original organism	Species	Mutant name	Protein	Phenotype	Source/ref.
G84C	G83C	G84C	*Schizosaccharomyces pombe*	*swo1–25 (swo1–26)*	SpHSP90	Temperature sensitivity; impaired glucose repression of fbp1(+) transcription; suppresses cell cycle arrest caused by overexpression of wee1 (+); advances mitosis; reduces the stability of the client protein Wee1	17, 18
T86E	T85E	T87E	*Triticum aestivum*		TaHSP90	Reduced binding to the CS domain of SGT1	1
K87E	K86E	K86E	*Saccharomyces cerevisiae*		ScHSP82	Reduced binding to the CS domain of SGT1	1
K87E	K86E	K88E	*Triticum aestivum*		TaHSP90	Reduced binding to the CS domain of SGT1; genetically suppresses AtSGT1a E223K mutant	1
A88E	A87E	S89E	*Triticum aestivum*		TaHSP90	Reduced binding to AtSGT1a	19
D89R	E88R	D90R	*Triticum aestivum*		TaHSP90	Reduced binding to the CS domain of SGT1 and AtRAR1	1
V91T	I90T	V92T	*Triticum aestivum*		TaHSP90	Reduced binding to AtSGT1a	19
N92R	N91R	N93R	*Triticum aestivum*		TaHSP90	Reduced binding to the CS domain of SGT1	1
G95E	G94E	G95E	*Arabidopsis thaliana*	*Athsp90.2–1*	AtHSP90.2	Loss of RPM1 function and accumulation; fully penetrant phenotype; loss of ATPase activity; loss of dimerization; loss of RAR1 interaction; loss of SGT1 interaction	16, This study
A98I	A97I	A97I	*Saccharomyces cerevisiae*		ScHSP82	Reduced levels of hormone binding by the ER	20
A98T	A97T	A97T	*Saccharomyces cerevisiae*		ScHSP82	Temperature sensitive	21
R99A	K98A	K106A	*Homo sapiens*		hHSP90 α	Reduced binding to GA	22
R99E	K98E	R100E	*Triticum aestivum*		TaHSP90	Enhanced binding to the CS domain of SGT1	1
S100A	S99A	S113A	*Homo sapiens*		hHSP90 α	Abolishes binding to geldanamycin (GA) and ATP	23
S100F	S99F	S100F	*Arabidopsis thaliana*	*Athsp90.2–2, -4*	AtHSP90.2	Loss of RPM1 function and accumulation; partially penetrant phenotype; loss of ATPase activity; loss of dimerization; normal RAR1 interaction; loss of SGT1 interaction	16, this study
G101D	G100D	G113D	*Gallus gallus*		HSP90α	Does not bind to novobiocin	24
T102I	T101I	T101I	*Saccharomyces cerevisiae*		ScHSP82	Temperature sensitivity; decreases the growth rates on media containing 0.05% maltose; reduced maltose induction; shortens the half-life of the client protein Mal63p which is a MAL activator; reduced levels of hormone binding by the estrogen receptor (ER); reduced activity of substrate Hap1; reduced GR activity; reduced formation of CBF3-centromere DNA; hypersensitive to GA and RA; enhanced interaction with cdc37; reduced nucleotide binding; reduced ATPase acitivity; diminished N-terminal dimerization	2, 4–7, 20, 21, 25–28
T102I	T101I	T101I	*Saccharomyces cerevisiae*		ScHSC82	Temperature sensitive growth defect; reduced GR and v-Src activities; reduced ATPase activity; reduced v-Scr accumulation; reduced interactions with Sba1 and Cpr6	8
A108N	A107N	A107N	*Saccharomyces cerevisiae*		ScHSC82	Reduced interaction with Sti1	8
A132D	A131D	A133D	*Drosophila melanogaster*		HSP83	Affected localization of nanos and pgc mRNA	29

Mutation position in reference to AtHSP90.2	Mutation position in reference to ScHSP90	Mutation position in original organism	Species	Mutant name	Protein	Phenotype	Source/ref.
D144R	D143R	D143R	*Saccharomyces cerevisiae*		ScHSP82	Reduced binding to the CS domain of SGT1	1
D144R	D143R	D145R	*Triticum aestivum*		TaHSP90	Reduced binding to the CS domain of SGT1 and AtRAR1	1
E145R	E144R	E146R	*Triticum aestivum*		TaHSP90	Reduced binding to the CS domain of SGT1	1
G155D	G154D	G155D	*Schizosaccharomyces pombe*	*swo1-w1*	SpHSP90	Sensitive to stressful growth conditions; mitotic defects	17, 18
G171D	G170D	G170D	*Saccharomyces cerevisiae*		ScHSP82	Temperature sensitivity; abolishes the activity of protein kinase Gcn2; increases HSF-dependent gene expression; abolishes the high affinity ligand binding conformation of human androgen receptor (AR); reduced levels of hormone binding by the ER; decreased activity of $p60^{V\text{-}src}$; reduced GR activity at high temperature; declines in telomere length; loses p23 binding and ATPase activity; abolishes the accumulation of Gcn2; reduced telomerase DNA binding	3, 4, 6, 20, 21, 27, 30–33
K265A	K274A	K294A	*Homo sapiens*		hHSP90 α	Mimic acetylated; reduced binding to cochaperon p23 and client protein ErbB2	34
K265Q	K274Q	K294Q	*Homo sapiens*		hHSP90 α	Mimic acetylated; reduced binding to Cochaperone p23 and client protein ErbB2	34
K265R	K274R	K294R	*Homo sapiens*		hHSP90 α	Unacetylated	34
W268G	W277G	W297G	*Homo sapiens*		hHSP90 α	Blocks self-oligomerization	35
W291A	W300A	W296A	*Saccharomyces cerevisiae*		ScHSC82	Reduced interaction with Sti1	8
W291A	W300A	W300A	*Saccharomyces cerevisiae*		ScHSP82	Temperature sensitive growth defect; reduced GR activity; enhanced v-Scr activity; reduced binding to Aha1; increased v-Scr accumulation	(2
E303K	E312K	E317K	*Drosophila melanogaster*	*Esev)3A e6D*	HSP83	Lethality; reduced Raf kinase activity; affected localization of nanos and pgc mRNA; reduced binding to Raf	14, 15, 29
G304N	G313N	G313N	*Saccharomyces cerevisiae*		ScHSP82	Affects all receptor types tested; temperature sensitivity; decreased growth rates; constitutive expression of transcription factor Gcn4; reduced levels of hormone binding by the ER; reduced activity of substrate Hap1; defective pheromone-signaling; decreases GR ligand binding activity; unstable aporeceptor complexes; reduced accumulation of substrate proteins Ste7 and Ste11	22, 28, 32, 36, 37
G304N	G313N	G329N	*Gallus gallus*		HSP90α	Affects interacting with HSP90 accessory proteins	38
G304S	G313S	G313S	*Saccharomyces cerevisiae*		ScHSP82	Impairs AhR signaling; reduced activity of substrate Hap1 and GR; temperature sensitivity	3, 4, 26
F320A	F329A	F325A	*Saccharomyces cerevisiae*		ScHSC82	Reduced interaction with Sti1	8
L334P	L343P	L338P	*Schizosaccharomyces pombe*	*git10–201*	SpHSP90	CAMP signaling defect; impaired glucose repression of fbp1(+) transcription	17
R337C	R346C	R337C	*Arabidopsis thaliana*	A*thsp90.2–8*	AtHSP90.2	Restoration of NB-LRR function and accumulation in a rar1 mutant; loss of ATPase activity; decreased dimerization; loss of RAR1 interaction; loss of SGT1 interaction	This study

Mutation position in reference to AtHSP90.2	Mutation position in reference to ScHSP90	Mutation position in original organism	Species	Mutant name	Protein	Phenotype	Source/ref.
F340A	F349A	F345A	*Saccharomyces cerevisiae*		ScHSC82	Reduced interaction with Sba1 and Cpr6	8
P350A	P359A	P379A	*Homo sapiens*		hHSP90 α	Blocks self-oligomerization	35
F355A	F364A	F384A	*Homo sapiens*		hHSP90 α	Blocks self-oligomerization	35
E363K	E372K	E377K	*Drosophila melanogaster*	*9J1*	HSP83	Reduced Raf kinase activity	15
L367D	L376D	L372D	*Saccharomyces cerevisiae*		ScHSC82	60-fold reduction of ATP hydrolysis	9
L367S	L376S	L392S	*Gallus gallus*		HSP90α	Reduced binding activity to p23	39
I369N	L378N	L374N	*Saccharomyces cerevisiae*		ScHSC82	3-fold reduction of ATP hydrolysis	9
R371A	R380A	R376A	*Saccharomyces cerevisiae*		ScHSC82	Reduced interaction with Sba1; 6.7 fold reduction of ATP hydrolysis	8, 9
E372K	E381K	E381K	*Saccharomyces cerevisiae*		ScHSP82	Impairs AhR signaling; temperature sensitivity; reduced GR activity; temperature sensitive growth defect; reduced GR and v-Src activities; reduced GR and v-Scr accumulations	2–4
E372K	E381K	E377K	*Saccharomyces cerevisiae*		ScHSC82	Reduced interaction with Sti1	8
E422K	E431K	E431K	*Saccharomyces cerevisiae*		ScHSP82	Affects glucocorticoid receptor (GR) signaling; Impairs Aryl hydrocarbon receptor (AhR) signaling; reduced levels of hormone binding by the ER; temperature sensitive growth defect; reduced v-Src activity; reduced ATPase activity; reduced GR accumulation	2, 3, 20, 36, 37
L448A	L457A	L477A	*Homo sapiens*		hHSP90 α	Blocks self-oligomerization; blocks binding to HtpGA	40
S476K	S485K	S485K	*Saccharomyces cerevisiae*		ScHSP82	Reduced levels of hormone binding by the ER	20
S476Y	S485Y	S485Y	*Saccharomyces cerevisiae*		ScHSP82	Temperature-sensitive growth defect; reduced GR and v-Src activities; reduced ATPase activity; reduced binding to p23 and AhaI; reduced v-Scr accumulation	2, 21
S476Y	S485Y	S481Y	*Saccharomyces cerevisiae*		ScHSC82	Reduced interactions with Sba1 and Cpr6	8
L482S	L491S	L487S	*Saccharomyces cerevisiae*		ScHSC82	Reduced interactions with Sba1 and Cpr6	8
E488A	E497E	E517A	*Homo sapiens*		hHSP90 α	Blocks self-oligomerization; blocks binding to HtpGA	40
ΔY491	ΔF500	ΔF492	*Podospora anserina*	*mod-E1*	Member of HSP90 family	Alters the sexual cycle and partially suppresses vegetative incompatibility	41
T516I	T525I	T525I	*Saccharomyces cerevisiae*		ScHSP82	Affects all receptor types tested; temperature sensitivity; decreased growth rates; constitutive expression of transcription factor Gcn4; decreases GR ligand binding activity; unstable aporeceptor complexes; reduced GR and v-Src activities; reduced ATPase activity; reduced binding to p23 and AhaI; reduced v-Scr accumulation	2, 21, 30, 36, 37
T516I	T525I	T521I	*Saccharomyces cerevisiae*		ScHSC82	Reduced interactions with Sba1 and Cpr6	8
T516I	T525I	T541I	*Gallus gallus*		HSP90α	Affects interacting with HSP90 accessory proteins	38
K532Q	R540Q	M553Q	*Homo sapiens*		hHSP90β	Moderately enhances dimeric activity	42
E537A	K545A	T566A	*Homo sapiens*		hHSP90 α	Decreased dimeric activity	42
E537T	K545T	A558T	*Homo sapiens*		hHSP90β	Enhances dimeric activity	42
E537V	K545V	A558V	*Homo sapiens*		hHSP90β	Enhances dimeric activity	42
E537I	K545I	A558I	*Homo sapiens*		hHSP90β	Enhances dimeric activity	42
E537R	K545R	A558R	*Homo sapiens*		hHSP90β	Enhances dimeric activity	42
E537Y	K545Y	A558Y	*Homo sapiens*		hHSP90β	Moderately enhances dimeric activity	42
S560C	S568C	S574C	*Drosophila melanogaster*	*E(sev)3A e3A*	HSP83	Lethality	14
D565T	D537T	S586T	*Homo sapiens*		hHSP90β	Moderately enhances dimeric activity	42
T578F	S586F	S592F	*Drosophila melanogaster*	*E(sev)3A e6A*	HSP83	Lethality; affected localization of nanos and pgc mRNA	14, 29

Mutation position in reference to AtHSP90.2	Mutation position in reference to ScHSP90	Mutation position in original organism	Species	Mutant name	Protein	Phenotype	Source/ref.
A579T	A587T	A587T	*Saccharomyces cerevisiae*		ScHSP82	Temperature sensitivity; reduced GR activity; hypersensitive to GA and RD; reduced activity of substrate Hap1	4, 7, 26
A579T	A587T	A583T	*Saccharomyces cerevisiae*		ScHSC82	Reduced interactions with Sba1 and Cpr6	8
S600A	S608M	A629M	*Homo sapiens*		hHSP90 α	Decreased dimeric activity	42
S600A	S608A	M621A	*Homo sapiens*		hHSP90β	Enhances dimeric activity	42
S600W	S608W	M621W	*Homo sapiens*		hHSP90β	Moderately enhances dimeric activity	42
S600V	S608V	M621V	*Homo sapiens*		hHSP90β	Moderately enhances dimeric activity	42
D615A	K623A	E644A	*Homo sapiens*		hHSP90 α	Increased chaperon activity	43
D622A	D630A	E651A	*Homo sapiens*		hHSP90 α	Decreased chaperon activity; inhibited binding to Hop	43
D624A	G632A	D653A	*Homo sapiens*		hHSP90 α	Decreased chaperon activity; abolished binding to Hop	43
L635I	K644I	V656I	*Homo sapiens*		hHSP90β	Enhances dimeric activity	42
E639A	E648A	E668A	*Homo sapiens*		hHSP90 α	Increased chaperon activity	43
A641F	A650F	S655F	*Drosophila melanogaster*	*E(sev3A e4A*	HSP83	Lethality	14
L649R	L658R	L654R	*Schizosaccharomyces pombe*	*swo1–21*	SpHSP90	Temperature- sensitive growth defect; impaired glucose repression of fbp1(+) transcription	17
F655A	F664A	F660A	*Saccharomyces cerevisiae*		ScHSC82	Reduced interactions with Sba1 and Cpr6	8
E685A	E698A	E720A	*Homo sapiens*		hHSP90 α	Abolished binding to PP5 and FKBP52	43
D687A	P700A	D722A	*Homo sapiens*		hHSP90 α	Abolished binding to PP5 and FKBP52	43
A688A	A701A	D723A	*Homo sapiens*		hHSP90 α	Abolished binding to PP5 and FKBP52	43
D689A	D702A	D724A	*Homo sapiens*		hHSP90 α	Abolished binding to PP5 and FKBP52	43
E696A	E706A	E729A	*Homo sapiens*		hHSP90 α	Abolished binding to TPR proteins	43
E697A	E707A	E730A	*Homo sapiens*		hHSP90 α	Abolished binding to TPR proteins	43
D699A	D709A	D732A	*Homo sapiens*		hHSP90 α	Abolished binding to TPR proteins	43
Changes involving 2 or more amino acids							
T23F/R371A	T22F/R380A	T22F/R376A	*Saccharomyces cerevisiae*		ScHSC82	1.6-fold reduction of ATP hydrolysis	9
F24A/R371A	V23A/R380A	V23A/R376A	*Saccharomyces cerevisiae*		ScHSC82	20-fold reduction of ATP hydrolysis	9
Y25A/R371A	Y24A/R380A	Y24A/R376A	*Saccharomyces cerevisiae*		ScHSC82	24-fold reduction of ATP hydrolysis	9
S219A/E239A	P218A/E249A	S226A/S255A	*Homo sapiens*		hHSP90β	Increased transcription activity of AhR gene; phosphorylation defect; enhanced interaction with AhR; reduces the resistance of mouse cell to cytochrome c; inhibits phosphorylation; enhances binding to client protein Apaf-1	44, 45
F320A/L322A/F323A	F329A/L331A/F332A	F329A/L331A/F332A	*Saccharomyces cerevisiae*		ScHSP82	Reduced GR and v-Src activities	2
R337A/R338A	R346A/R347A	R362A/R363A	*Gallus gallus*		HSP90α	Abolishes binding to p23	39
L369N/R371A	L378N/R380A	L374N/R376A	*Saccharomyces cerevisiae*		ScHSC82	24-fold reduction of ATP hydrolysis	9
A568T/V571K	A576T/R579K	A576T/R579K	*Saccharomyces cerevisiae*		ScHSP82	Affects all receptor types tested; temperature sensitivity; decreased growth rates; decreases GR ligand binding activity; unstable aporeceptor complexes	36, 37
I584A/M585A	I592A/M593A	I588A/M589A	*Saccharomyces cerevisiae*		ScHSC82	Reduced interactions with Sba1 and Cpr6	8
L636S/L637S	L645S/L646S	L665S/L666S	*Homo sapiens*		hHSP90 α	Blocks self-oligomerization and binding to client protein	46
L642S/L643S	L651S/L652S	L647S/L648S	*Saccharomyces cerevisiae*		ScHSC82	Reduced interactions with Sba1 and Cpr6	8
L642S/L643S	L651S/L652S	L671S/L672S	*Homo sapiens*		hHSP90 α	Blocks self-oligomerization and binding to client protein	46
E696A/E697A	E706A/E707A	E725A/E726A	*Gallus gallus*		HSP90α	Affects interacting with HSP90 accessory proteins	38

Neither the *rsp* phenotype nor the *rsp* allele mutations have been previously observed. This is a comprehensive list of previously-identified HSP90 mutations identified from all organisms in relation to HSP90.2 amino acid sequence. Mutations are given in relation to *At*HSP90.2 and *Sc*HSP82 sequence and the sequence of the originally-identified mutation. Genetic and biochemical characterization of each mutation is also given. Truncations and large deletions have been omitted.

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