

Additional file 9 – Amino acid alignment of substrate recognition sites (SRS) and functional domains for hydroxylation activity (CR1) in plant *F3'5' Hs*

Amino acid positions crucial for 3' vs. 3',5'-hydroxylation in CR1 and SRS6 are indicated by black arrows; significant amino acid substitutions in grapevine *F3'5' Hs* are in green background.

Relevant amino acid substitutions within domains putatively involved in substrate recognition are highlighted in grapevine *F3'5' Hs* by blue background when they are unique with respect to all other plant *F3'5' Hs* or when they are shared exclusively with either monocot *F3'5' Hs* or other plant *F3' Hs*, as possible remnants of ancestral transition stages in the evolution of dicot *F3'5' Hs*.

	SRS1	CR1	SRS2	SRS4	SRS5	SRS6
		↓ ↓				↓ ↓
<i>Vitis_F35Ha</i>	-----	-----	-----	LLNLFTAGTDTSSSV	HPSTPLNLPR	-----
<i>Vitis_F35Hh</i>	-----	-----	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hc</i>	NRPPNAGATLLAYHAQDMVFADY	ANMIGQVILS	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	-----
<i>Vitis_F35He</i>	NRPPNAGATLLAYHAQDMVFADY	-----	-----	-----	-----	-----
<i>Vitis_F35Hi</i>	NRPPNAGATLLAYHAQDMVFADY	ANMIGQVILS	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hj</i>	NRPPNAGATLLAYHAQDMVFADY	ANMIGQVILS	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hd</i>	NRPPNAGATLLAYHAQDMVFADY	ANMIGQVILS	VELMTSAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hk</i>	SRPPNAGATLLAYHAQDMVFADY	ANMIGQVILS	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hf</i>	NRPPNAGATHLAYDAQDMVFADY	ANMIGQVILS	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hl</i>	NRPPNAGATHLAYDAQDMVFADY	ANMIGQVILS	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hg</i>	NRPPNAGATHLAYDAQDMVFADY	ANMIGQVILS	VELMTSAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hm</i>	NRPPNAGATHLAYGAQDMVFADY	ANMIGQVILS	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hn</i>	NRPPNAGATHLAYGAQDMVFADY	ANMIGQVILS	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hb</i>	NRPPNAGATLLAYHAQDMVFADY	ANMIGQVILS	VELMTSAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Ho</i>	NRPPNAGATSHLYGQDMVFADY	ANMIGQVILS	VELMTSAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Cammelia_F35H</i>	NRPPNAGATHLAYGAQDMVFADY	ANMIGQVILS	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Carica_F35H</i>	NRPPNAGATHLAYDAQDMVFADY	ANMIGQVILG	VELMTTAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Glycine_F35H</i>	NRPSNAGATHLAYDARDMVFADY	ANMIGQVILS	VELMTVAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Populus_F35Ha</i>	NRPPNAGATHLAYNAQDMVFADY	ANMIGQVILS	VELMTSAGL	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Populus_F35Hb</i>	NRPIDGGPTHLAYNAQDMVFADY	ANMIGQVILS	VELMTSGG	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Gossypium_F35H</i>	NRPSNAGATHIAYNSQDMVFADY	ANMIGQVILS	VELMTSAGL	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Viola_F35H</i>	NRPPNAGASHLAYGAQDLVFADY	ANMIGQVILS	VELMTSAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Rhododendron_F35H</i>	NRPPNAGATHLAYNSQDMVFADY	ANMIGQVILG	VELMTSAGL	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Eustoma_F35H</i>	NRPPNAGATHLAYNAQDMVFADY	ANMIGQVILS	VELMTVAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Gentiana_F35H</i>	NRPPNAGATHLAYNAQDMVFADY	ANMIGQVILS	VELMTSAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vinca_F35H</i>	NRPPNAGATHLAYGAQDMVFADY	ANMIGQVILS	VELMTSAGL	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Nierembergia_F35H</i>	NRPPNAGATHLAYNAQDMVFADY	ANMIGQVILS	VELMTVAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Petunia_F35H</i>	NRPPNAGATHLAYNAQDMVFADY	ANMIGQVILS	VELMTIAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Solanum_m_F35H</i>	NRPPNAGATHMAYNAQDMVFADY	ANMIGQVILS	VELMTVAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Solanum_t_F35H</i>	NRPPNAGATHLAYNAQDMVFADY	ANMIGQVILS	VELMTVAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Lycianthes_F35H</i>	NRPPNAGATHLAYDAQDMVFADY	ANMIGQVILS	VELMTVAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Torenia_F35H</i>	NRPPNAGATHLAYGAQDMVFADY	ANMIGQVILS	VELMTSAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Verbena_F35H</i>	DRPPNAGATLLAYNAQDMVFADY	ANMIGQVILS	VELMTSAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Campanula_F35H</i>	NRPIDGGPTLYLAYNAQDMVFADY	ANMIGQVILS	MELMRMAGL	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Lobelia_F35H</i>	NRPLEGGPTHLAYNAQDMVFADY	ANMIGQVILS	MELMRVAGL	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Delphinium_F35H</i>	NRPTDAGATHIAYNSQDMVFADY	ANMIGQVILS	IELMTSAGL	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hp</i>	NRPPNAGATHIAYDAQDMVFADY	ANMIGQVILS	VELMTSAGL	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Picea_F35H</i>	NRPPNAGATYIAYDSQDMVFADY	ANMIGQVILS	VELMTSAGY	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Phalaenopsis_F35H</i>	DRPLDIISKQVSYNGQNMVFADY	ANMIGQVILS	KELLVGGGA	FISDLFVAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Dendrobium_F35H</i>	DRPSVTSKEISYNGQNMVFADY	ANMIGQVILS	KELLVGGGA	FISDLFVAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Sorghum_F35Ha</i>	NRPAVASAADITYGQNMVFADY	ANMIGQVILS	VSLLTGAGL	LIFDMFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Zea_F35H</i>	NRPAVASAADITYGQNMVFADY	ANMIGQVILS	VSLMTGTGL	LIF-----	HPSTPLNLPR	MDEAFGLAL
<i>Sorghum_F35Hb</i>	NRPAVASAADITYRRQNMVFADY	ANMIGQVILS	VSLLTGTGM	LIFDMFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Oryza_F35H</i>	NRPAVASAVDITYNYQDMVFADY	ANMIGQVILS	VSLLTGAGL	LIFDMFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Ha</i>	NRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Vitis_F35Hb</i>	NRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Populus_F3H</i>	SRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Hieracium_F3H</i>	SRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Osteospermum_F3H</i>	SRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Gerbera_F3H</i>	DRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Lobelia_F3H</i>	NRPPNSGAVHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Ipomoea_F3H</i>	NRPPNSGAEHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Petunia_F3H</i>	SRPPNSGAEHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Gentiana_F3H</i>	NRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Torenia_F3H</i>	NRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Perilla_F3H</i>	SRPPNSGAEHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Arabidopsis_F3H</i>	SRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Matthiola_F3H</i>	SRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Carica_F3H</i>	SRPPNSGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Glycine_F3H</i>	SRPPNAGAKYIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Pelargonium_F3H</i>	SRPPNAGAKHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Allium_F3H</i>	NRPPNSGAEHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Tricyrtis_F3H</i>	NRPPNSGAEHIAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Sorghum_F3Ha</i>	NRPPNSGAEHVAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Sorghum_F3Hb</i>	NRPPNSGAEHVAYNYQDMVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Zea_F3Ha</i>	NRPPNSGAEHVAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Oryza_F3Ha</i>	NRPPNSGAEHVAYNYQDLVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Sorghum_F3Hc</i>	CRPPNSGELMAYNYQDMVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Zea_F3Hb</i>	SRPPNSGELMAYNYQDMVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL
<i>Oryza_F3Hb</i>	SRPPNSGELMAYNYQDMVFADY	TNALGRVILG	VELMTVAGV	LLNLFTAGTDTSSSV	HPSTPLNLPR	MDEAFGLAL