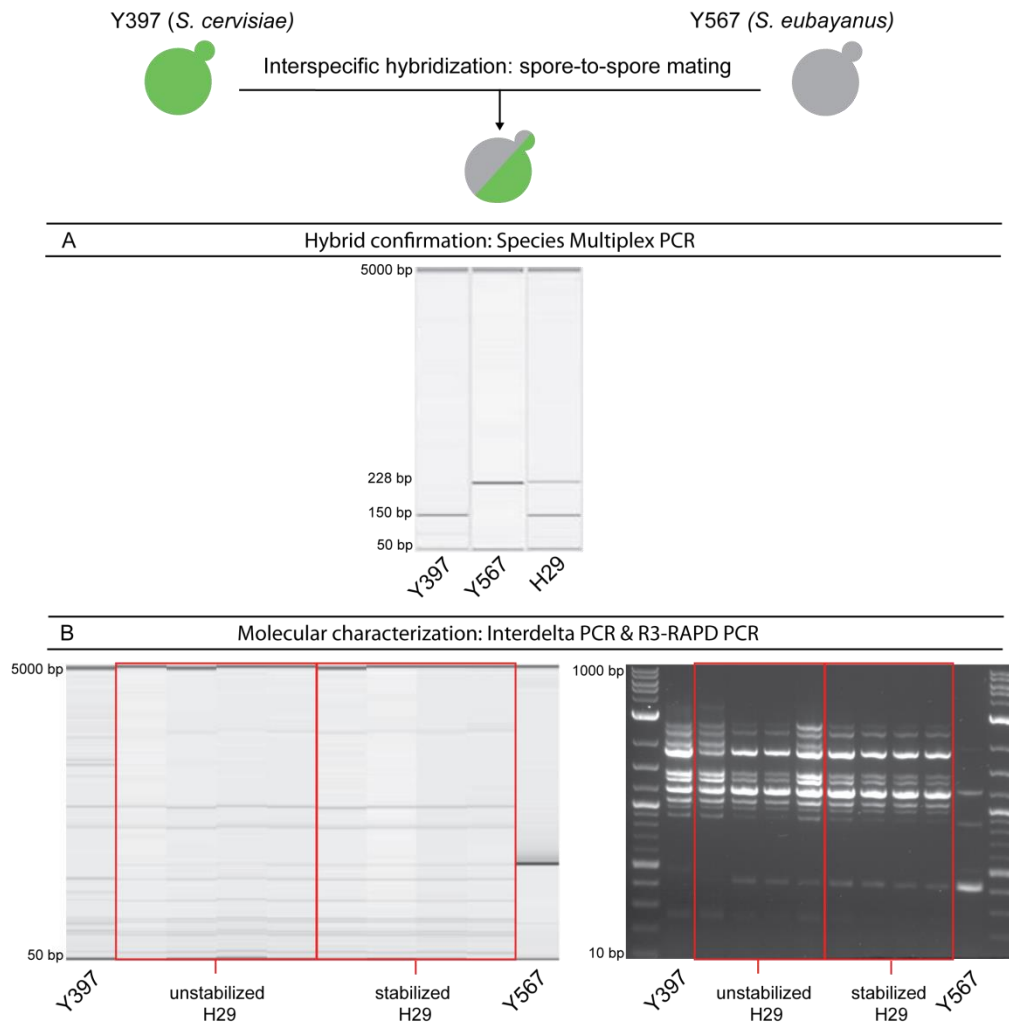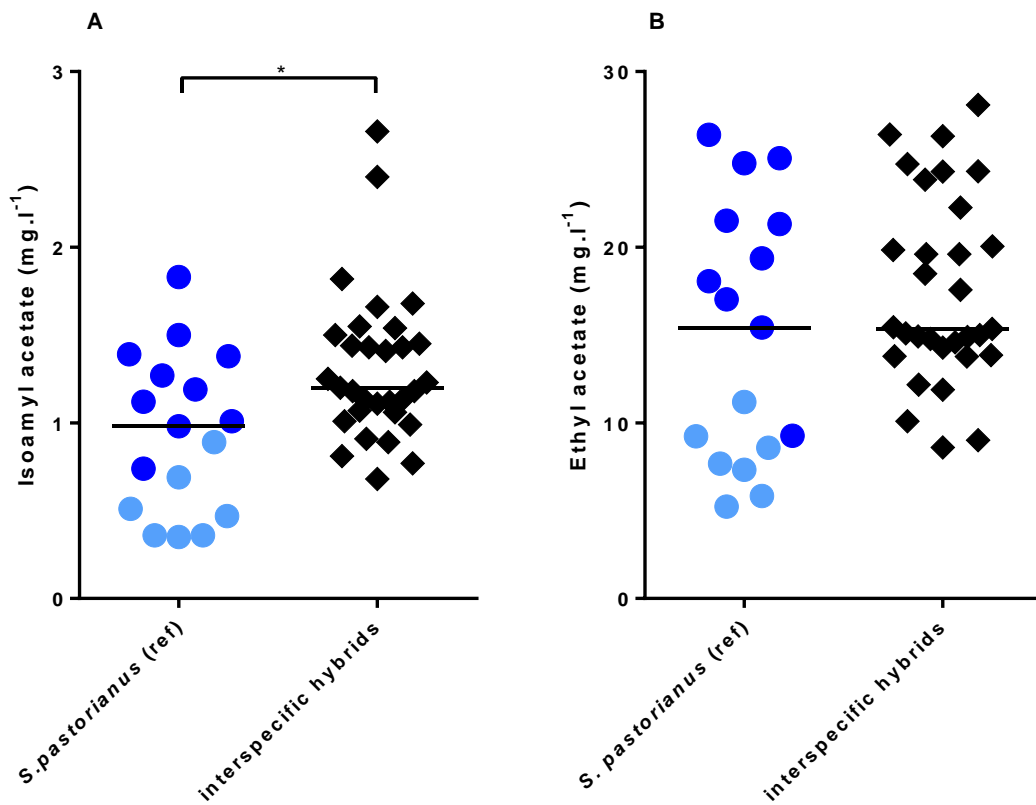**Fig. S1 Example of performed molecular analyses on hybrid nature and stability check of hybrid H29.** (A) Hybrid nature of possible hybrids was assessed based on the species multiplex PCR (see Materials and Methods for details). Two bands (each originating from both parents), confirm the hybrid nature of H29 (ale-type *S. cerevisiae* Y397 X *S. eubayanus* Y567). (B) Hybrid genome stability was assessed performing two DNA-fingerprints before (lanes 2-5) and after the applied stabilization protocol (lanes 6-9) and compared with its respective parental strains (interdelta analysis (left) and RAPD-R3 (right); see Materials and Methods for details). Interdelta profiles of hybrids before and after stabilization were the same. RAPD-R3 fingerprints of hybrid

H29 before stabilization showed differences, whereas after the stabilization protocol no heterogeneity in hybrids was detected. This indicates that the newly developed hybrids after stabilization were stable.

Fig. S2 Comparison of (A) isoamyl acetate and (B) ethyl acetate production of developed interspecific hybrids and 17 reference *S. pastorianus* strains. The production of isoamyl acetate and ethyl acetate for 7 Saaz type (light blue circles) and 10 Frohberg type (dark blue circles) *S. pastorianus* strains is compared to the production of the developed interspecific hybrids. The median aroma production is depicted as a black horizontal line. The developed interspecific hybrids produced a significant higher amount of isoamyl acetate compared to the 17 *S. pastorianus* strains (Mann-Whitney test; p< 0.05 (*))

Table S1 overview aroma and ethanol production from lab scale lager beer fermentation tests. DNA content is represented as times the average DNA content of the haploid reference yeasts ("/" means not measured). Quantified yeast-related aroma compounds are represented as concentrations (mg.l^{-1}), 4-VG production capacity is qualitatively indicated (+ and -) and the measured final ethanol content as volume percentages. Lastly, the used score legend for flavors during sensory analysis was: VS = very slightly; S = slightly; V = very; N =neutral; SW = sweet; FR = fruity; FL = floral; SULF = sulphury; SOLV = solvent-like; FRESH = fresh.

	DNA content	Acetaldehyde (mg.l^{-1})	Ethyl acetate (mg.l^{-1})	Ethyl propionate (mg.l^{-1})	Isobutyl acetate (mg.l^{-1})	Ethyl Butyrate (mg.l^{-1})	Propanol (mg.l^{-1})	Isobutanol (mg.l^{-1})	Isoamyl acetate (mg.l^{-1})	Butanol (mg.l^{-1})	Isoamyl alcohol (mg.l^{-1})	Phenyl ethyl acetate (mg.l^{-1})	Phenyl ethanol (mg.l^{-1})	ethanol (%vv^{-1})	Potential to produce 4-VG	Sensory analysis
H1	1,67	22.36	13.78	0.25	0.09	0.11	6.79	12.08	1.12	0.64	48.78	0.70	6.97	4.78	+	SW
H2	1,39	23.18	11.88	0.22	0.07	0.10	5.72	9.01	1.13	0.52	38.21	0.81	9.00	4.54	+	SW
H3	1,69	22.64	14.77	0.33	0.07	0.08	8.85	14.19	0.91	0.75	71.91	0.88	12.09	5.56	+	SULF
H4	1,64	29.30	13.80	0.28	0.08	0.12	7.07	10.19	1.20	0.69	46.94	1.02	11.87	3.74	+	VSSW
H5	1,75	20.94	20.05	0.43	0.08	0.13	6.70	10.56	1.66	0.71	52.26	1.22	17.85	3.88	+	SW,FRESH
H6	2,07	19.59	13.86	0.28	0.07	0.10	6.39	9.91	1.18	0.00	37.85	0.76	9.78	4.48	+	N
H7	1,72	19.33	15.00	0.26	0.10	0.09	7.17	12.93	1.25	0.76	56.07	1.28	14.01	3.78	+	SSW
H8	1,91	19.56	9.02	0.29	0.06	0.12	6.62	11.97	0.77	0.86	53.93	0.75	13.25	3.76	+	FR,SW
H9	1,59	19.84	8.60	0.24	0.08	0.06	6.49	14.16	1.07	0.74	57.72	1.09	32.00	2.96	+	SW,SFL
H10	1,85	23.68	12.18	0.32	0.06	0.08	6.91	10.90	0.89	0.68	46.49	0.87	10.07	3.96	+	SW,SSULF
H11	1,68	14.67	10.09	0.30	0.05	0.06	5.90	8.78	0.68	0.67	41.59	0.91	12.02	4.62	+	N, SSULF

H12	2,91	6.97	14.89	0.16	0.07	0.11	5.62	9.27	1.06	0.19	36.12	0.74	8.79	5.30	+	SW,FR
H13	3,62	19.69	15.35	0.28	0.08	0.18	6.25	10.98	1.23	0.26	39.67	0.86	11.18	5.50	+	SSW,SFL
H14	2,36	9.21	19.84	0.48	0.09	0.16	9.92	14.82	1.54	1.32	75.96	1.10	27.37	4.70	+	SW, SSULF
H15	3,15	16.83	22.26	0.25	0.07	0.19	9.32	8.27	1.43	1.02	44.58	0.55	5.89	6.20	+	N,SULF
H16	2,84	14.93	24.73	0.34	0.09	0.14	8.83	10.91	1.44	0.84	48.40	0.67	6.20	5.18	+	SW,FL
H17	3,05	14.03	26.42	0.36	0.09	0.22	7.76	8.81	1.82	0.85	37.72	0.89	6.96	5.72	+	SSW,SFL
H18	3,43	20.91	18.49	0.29	0.09	0.21	7.94	9.45	1.45	1.03	44.23	0.69	7.92	5.32	+	N,SSULF
H19	3,10	16.76	15.10	0.24	0.07	0.10	6.14	8.19	1.18	0.79	39.79	0.54	6.48	5.16	+	SULF
H20	3,12	24.82	23.84	0.30	0.09	0.14	6.64	7.86	1.50	0.84	28.62	0.68	4.67	4.12	+	N,SSW
H21	3,56	24.50	14.92	0.16	0.06	0.14	7.34	8.18	1.11	0.89	40.04	0.62	7.38	4.50	+	SW,FL
H22	2,96	8.62	24.32	0.34	0.08	0.22	6.51	6.66	1.55	0.66	32.29	0.74	6.78	4.72	+	(S)FR
H23	2,85	19.34	19.61	0.39	0.06	0.14	8.20	8.64	1.13	1.13	44.99	0.67	7.30	5.32	+	SSW,SFR
H24	3,20	34.28	19.60	0.20	0.08	0.17	7.03	7.64	1.43	0.75	39.28	0.96	10.07	5.96	+	N, FR
H25	2,77	24.62	17.58	0.20	0.05	0.31	8.10	8.06	0.81	1.05	46.13	0.72	7.07	4.72	+	SSW,SFR
H26	3,01	23.16	14.58	0.53	0.05	0.25	6.36	7.00	1.01	1.03	41.61	0.80	7.95	5.08	+	SSW,FL
H27	3,03	16.59	14.28	0.26	0.06	0.08	5.89	7.61	0.99	0.76	39.20	0.80	7.89	4.28	+	N
H28	2,11	8.70	15.45	0.35	0.07	0.09	6.33	8.26	1.41	0.23	37.67	1.18	10.73	4.08	+	SW,FR
H29	2,58	14.24	26.32	0.46	0.14	0.19	9.38	12.96	2.66	0.86	73.77	2.15	41.02	6.12	+	FR, SW, FRESH
H30	2,24	22.63	24.31	0.40	0.10	0.14	7.01	7.72	1.68	0.77	34.97	1.12	10.18	4.76	+	FR, SW, FRESH
H31	3,23	22.50	28.11	0.36	0.12	0.20	5.97	8.11	2.40	0.86	38.41	1.31	9.83	4.78	+	FR, SW, FRESH
Y134	2,94	10.71	12.47	0.19	0.05	0.12	7.90	7.69	0.94	0.23	32.44	0.76	14.11	4.40	-	SFL,SSW, SOLV
Y184	1,77	20.21	7.61	0.16	0.05	0.04	7.54	11.65	0.66	0.55	40.77	0.70	31.83	3.10	-	SW,VFR
Y397	3,77	4.24	18.57	0.41	0.08	0.10	10.24	12.19	1.51	1.04	72.08	1.34	16.47	5.00	+	VFR,SSW
Y377	3,72	3.91	17.49	0.23	0.07	0.21	7.35	8.85	1.39	0.75	50.21	0.57	8.21	4.52	+	VSW
Y245	4,00	3.89	16.98	0.24	0.07	0.18	7.44	6.85	1.24	0.77	32.84	0.59	7.69	4.48	+	VFR,FL
Y470	1,55	13.42	7.30	0.16	0.05	0.04	6.13	9.08	0.64	0.62	36.76	0.58	14.16	2.92	-	SF,SSW
Y565	2,03	7.77	5.66	0.11	0.06	0.04	4.81	12.37	0.64	0.68	48.13	0.80	13.09	2.76	+	SSULF
Y567	1,59	16.58	7.36	0.20	0.07	0.05	5.68	14.96	0.69	1.01	56.04	0.87	17.55	2.38	+	SULF
GSY132	2,52	7.01	9.26	0.36	0.05	0.07	5.70	10.59	0.74	2.80	39.31	0.74	13.85	4.20	-	N
GSY 501	3,61	9.21	7.68	0.43	0.03	0.18	6.47	8.05	0.36	1.00	38.08	0.53	16.94	4.56	-	VSW,F,FR
GSY134	/	28.20	5.23	0.30	N.D.	0.06	4.82	6.30	0.36	0.16	35.33	0.74	18.17	3.50	-	VSSW, N
GSY133	/	8.37	5.84	0.39	0.03	0.12	5.28	7.28	0.35	0.16	30.04	0.49	22.77	4.48	-	N
GSY129	/	9.37	11.19	0.33	0.05	0.11	5.28	10.23	0.89	N.D.	38.62	0.61	13.04	4.46	-	VSSULF, N
GSY131	/	18.23	8.59	0.32	N.D.	0.10	4.97	5.57	0.51	0.20	34.98	0.64	22.17	6.10	-	VSSULF,N
GSY509	/	8.75	9.23	0.28	0.05	0.16	5.75	12.06	0.69	N.D.	43.82	0.79	8.94	5.56	-	N, VSSW
GSY137	/	13.20	7.33	0.32	N.D.	0.08	4.99	5.93	0.47	0.22	36.34	0.40	8.03	4.82	-	VSSULF, N
Y449	/	11.69	25.07	0.38	0.07	0.15	5.95	6.66	1.39	0.26	26.69	0.63	4.96	5.98	-	FR
Y473	/	11.73	24.78	0.57	0.09	0.09	9.09	11.14	1.38	0.90	40.28	1.55	12.59	4.70	-	N
Y453	/	7.12	19.36	0.46	0.06	0.10	8.60	8.35	1.19	0.55	35.37	1.20	9.30	5.96	-	VSSULF,N

Y454	/	9.46	21.32	0.41	0.09	0.09	7.61	9.38	1.50	0.74	37.62	1.46	9.33	5.98	-	N
GSY516	/	11.49	18.07	0.26	0.07	0.11	7.82	8.26	0.98	0.22	29.31	0.53	8.69	5.24	-	VSSULF,VSSW
GSY135	/	11.47	15.43	0.54	0.05	0.10	9.46	6.57	1.01	0.36	30.76	1.12	27.47	3.92	-	VSSW
Y472	/	11.78	24.60	0.48	0.08	0.12	6.71	6.13	1.28	0.22	24.54	0.90	5.63	6.94	-	N
GSY515	/	11.52	26.40	0.52	0.11	0.08	7.58	11.11	1.83	0.59	37.91	1.51	6.80	6.08	-	SW,FR
Y5	/	7.19	17.04	0.66	0.06	0.09	10.17	10.39	1.12	0.87	44.72	0.86	9.09	4.96	-	N, SFL

Table S2 obtained results Kruskal-wallis test and pair wise post hoc Dunn tests for comparison growth capacity of the generated interspecific hybrids with parental strains (Kruskal-Wallis test P-value or Benjamini- Hochberg corrected p-value < 0.05: $^{(*)}$ or < 0.01 : $^{(**)}$)

	Kruskal -wallis test		Dunn test with bh -corrected p-values								
			S. cerevisiae - hybrid			*S. eubayanus* - hybrid			*S. eubayanus* - *S. cerevisiae*		
	Chi-squared	P-value	Z	P-value	corrected P-value	Z	P-value	corrected P-value	Z	P-value	corrected P-value
4°C (15days)	16.12$^{(**)}$	0	3.25 $^{(**)}$	0.0006	0.0009	-2.02 $^{(*)}$	0.0215	0.0215	-3.58 $^{(**)}$	0.0002	0.0005
8°C (5days)	13.48 $^{(**)}$	0	2.82 $^{(**)}$	0.0024	0.0036	-2.06 $^{(*)}$	0.0195	0.0195	-3.38 $^{(**)}$	0.0004	0.0011
10°C (5 days)	10.98 $^{(**)}$	0	2.39 $^{(*)}$	0.0084	0.0125	-2.04 $^{(*)}$	0.0205	0.0205	-3.13 $^{(**)}$	0.0009	0.0026
16°C (2days)	8.62 $^{(**)}$	0.01	2.51 $^{(*)}$	0.0061	0.0182	-1.27	0.1017	0.1017	-2.5 $^{(**)}$	0.0061	0.0091
30°C (2days)	6.05 $^{(*)}$	0.05	0.89	0.1858	0.1858	2.37 $^{(*)}$	0.0089	0.0267	1.63	0.0516	0.0775
37°C (2days)	11.82 $^{(**)}$	0	-2.50 $^{(**)}$	0.0061	0.0092	2.1 $^{(*)}$	0.0180	0.0180	3.24 $^{(**)}$	0.0006	0.0018
41°C (2 days)	27.31 $^{(**)}$	0	-5.17 $^{(**)}$	0.0000	0.0000	0.26	0.3974	0.3974	3.05 $^{(**)}$	0.0011	0.0017

Table S3 obtained results Kruskal-wallis test and pair wise post hoc Dunn tests for comparison aroma production of the generated interspecific hybrids with Saaz- and Frohberg- type reference *S. pastoriuanus* strains (Kruskal-Wallis test P-value or Benjamini- Hochberg corrected p-value < 0.05: [*] or < 0.01 : [**])

| | Kruskal -wallis test | | Dunn test with bh -corrected p-values | | | | | | | | |
| | Chi-squared | P-value | hybrid - Frohberg | | | hybrid - Saaz | | | Saaz - Frohberg | | |
			Z	P-value	corrected P-value	Z	P-value	corrected P-value	Z	P-value	corrected P-value
acetaldehyde	15.39 [**]	0	-3.69 [**]	0.0001	0.0003	-2.08	0.0187	0.0281	-0.96	0.1694	0.1694
ethyl acetate	17.72 [**]	0	1.26	0.1040	0.1040	-3.66 [**]	0.0001	0.0002	4.04 [**]	0.0000	0.0001
ethyl propionate	11.54 [**]	0	3.39 [**]	0.0004	0.0011	0.99	0.1614	0.1614	1.66	0.0486	0.0729
isobutyl acetate	16.99 [**]	0	-0.37	0.3554	0.3554	-4.09 [**]	0.0000	0.0001	3.20 [**]	0.0007	0.0010
ethyl butyrate	6.52 [*]	0.04	-2.50 [**]	0.0062	0.0186	-1.03	0.1516	0.2274	-0.97	0.1657	0.1657
propanol	14.89 [**]	0	1.01	0.1561	0.1561	-3.43 [**]	0.0003	0.0005	3.65	0.0001	0.0004
isobutanol	4.34	0.11	-0.32	0.3757	0.3757	-2.08	0.0188	0.0563	1.53	0.0627	0.0941
isoamyl acetate	16.22 [**]	0	-0.3	0.3822	0.3822	-3.99 [**]	0.0000	0.0001	3.17 [**]	0.0008	0.0012
butanol	9.94 [*]	0.01	-0.99	0.1618	0.1618	-3.14 [**]	0.0009	0.0026	1.93	0.0265	0.0398
isoamyl alcohol	11.68 [**]	0	-2.85 [**]	0.0022	0.0065	-2.45 [**]	0.0072	0.0108	-0.03	0.4893	0.4893
phenyl ethyl acetate	11.17 [**]	0	1.22	0.1109	0.1109	-2.78 [**]	0.0027	0.0041	3.26 [**]	0.0006	0.0017
phenyl ethanol	4.03	0.13	0.17	0.4308	0.4308	1.99	0.0232	0.0695	-1.56	0.0590	0.0885

Table S4 Overview results sensory evaluation from pilot scale fermentation tests by professional tasting panel. Score legend: absent (/); very very slightly present (- - -); very slightly present (- -); slightly present (-); mildly present (- +); present (+); clearly present (++); predominant (+++).

		Y134	Y397	Y470	GSY501	H15	H29
Aroma	**malt**	grainy (-)	/	grainy (- -)	/	grainy (-)	/
	hoppy	/	/	grassy (+)	/	/	/
	esters	/	fruity (banana/pineapple) (++)	fruity (-)	fruity (pineapple) (++)	/	fruity (banana) (-)
	phenolic	/	/	/	/	/	/
Flavor	**Malt**	/	/	grainy (- -)	/	grainy (+)	/
	Hoppy	/	/	grassy (+)	/	/	/
	esters	fruity (apple/pear) (-)	fruity (banana/pineapple) (++)	fruity (- -)	fruity (pineapple) (++)	/	fruity (banana) (+)
	phenolic	/	/	/	/	/	/
other		sulphur (- +)	sulphur (+)/onion (+)	/	/	sulphur (- +)/ metallic (+)	sulphur (-)
taste/mouthfeel	**Bitterness intensity**	+	+	+	+	+	+
	bitterness quality	+++	+	+++	+ + +	+	+ + +
	sweet	-	-	- - -	- -	- -	- -
	body	-	-	-	-	-	+
	astringency	-	- -	- - -	- - -	- +	- - -
Overall impression	**appreciation**	+	- +	+	+	-	+
	complexity	- +	- +	--	- +	- +	+
	balance	+	- -	- +	+	- - -	- +