ELECTRONIC SUPPLEMENTARY INFORMATION

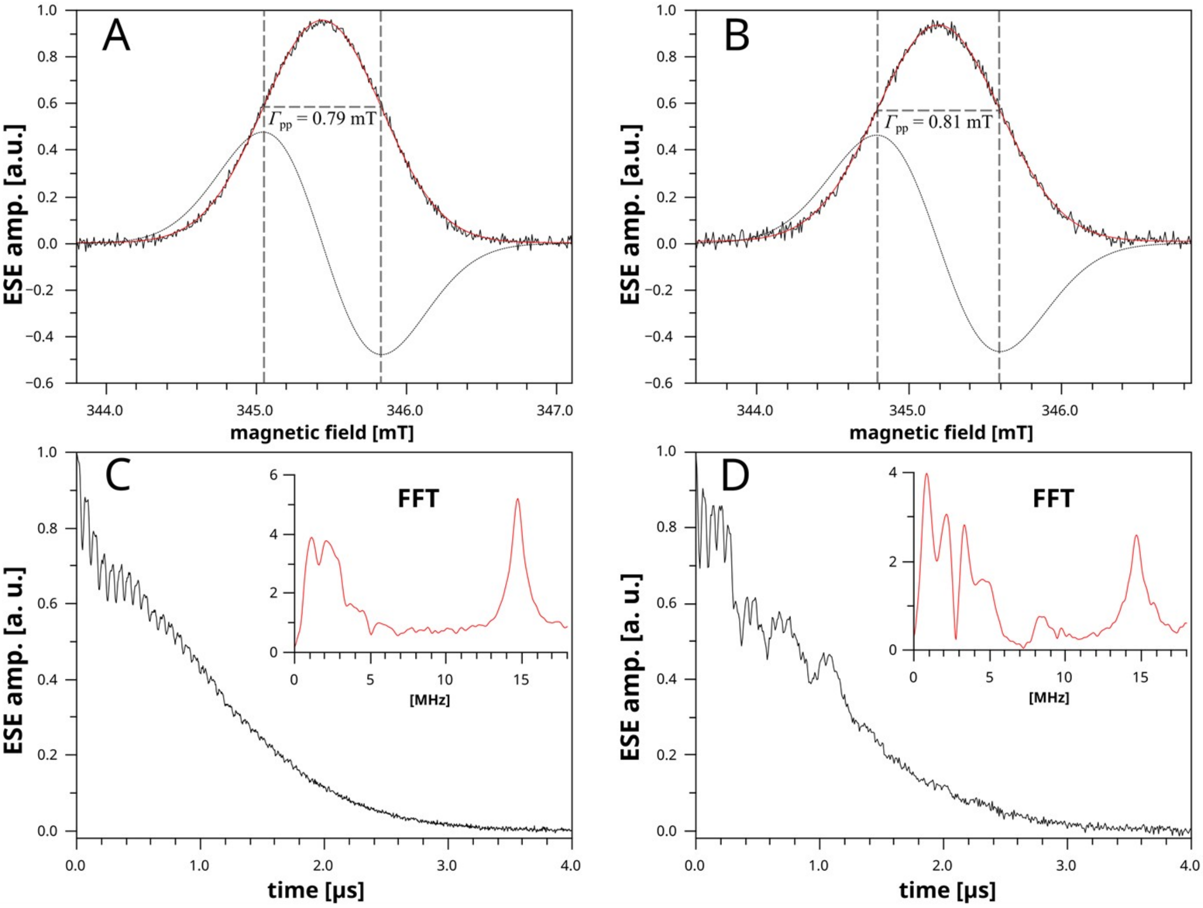

Fig. S1. Basic 2-pulse X-band Electron Spin Echo (ESE) experimental results of SQ_i. (*top*) EPR absorption-like spectra of SQ_i in WT and H217R mutant (A and B, respectively). Fitting gaussian profiles (red lines) gave the peak-to-peak linewidth of 0.79 mT and 0.81 mT for WT and H217R, respectively. The derivative (grey line) of the gaussian profile was shown for reference. (*bottom*) ESE decay traces with visibly distinct nuclear modulation patterns obtained for WT and H217R (C and D, respectively). The traces were measured at the centre of the SQ_i spectrum. The frequency spectra in the insets (i.e. Fourier transform of the time traces) show clear differences in the low frequency region (up to 5 MHz).

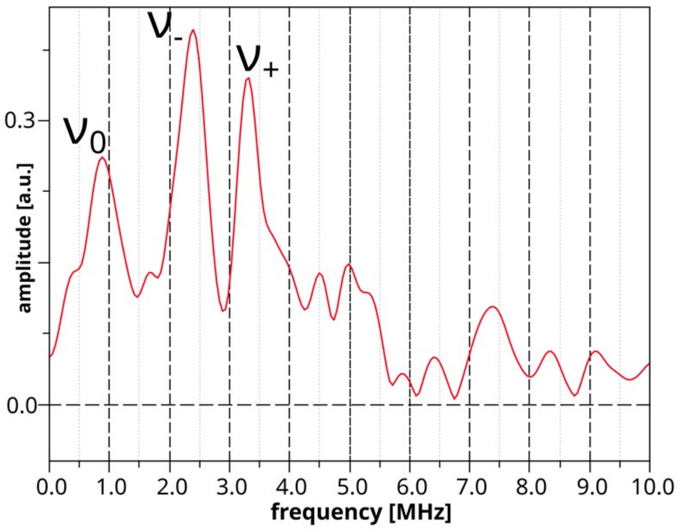

Fig. S2. X-band 3p-ESEEM spectrum of SQ$_i$ in H217R mutant. The frequencies of ν_0 = 0.8 MHz, ν_- = 2.4 MHz and ν_+ =3.2 MHz (i.e. $\nu_0+\nu_-=\nu_+$) are related to quadrupolar interaction transitions and indicate that the system is in cancelation conditions $a \sim 2\nu_I$ (^{14}N) ~ 2 MHz. The parameters were as follows: τ = 136 ns, $\pi/2$ and π: 16 ns and 32 ns.

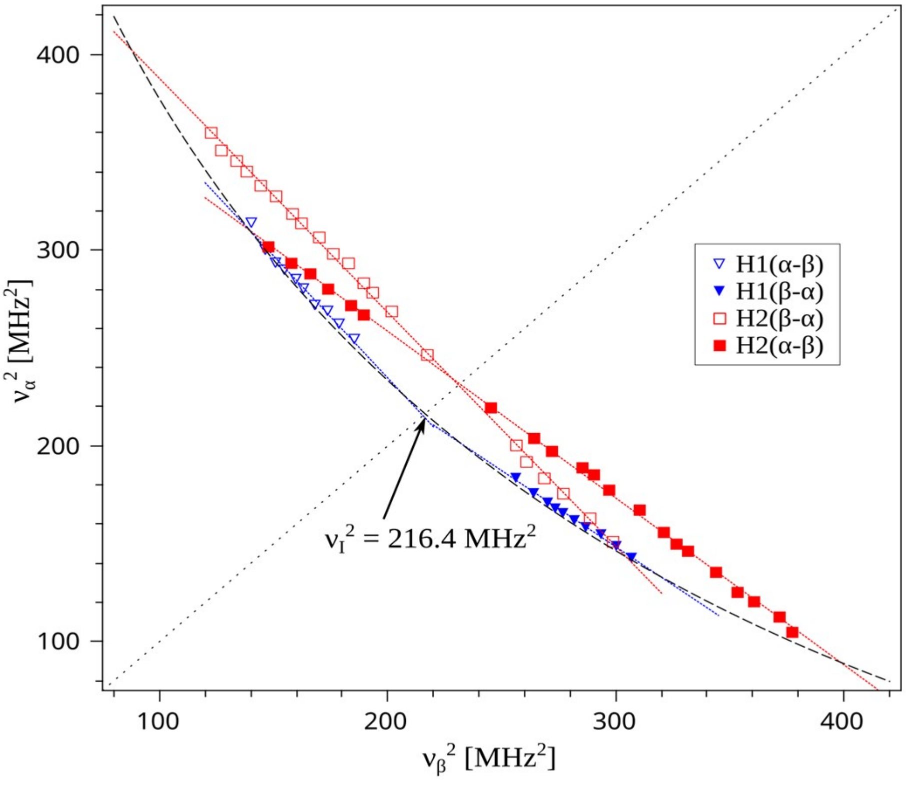

Fig. S3. Analysis of the proton HYSCORE spectrum of WT SQ$_i$ in squared frequency coordinates. In this representation the arcs in HYSCORE map form straight lines. The representative sets of points were picked from original HYSCORE map by aiming at the points of highest intensity in the respective ridges. For the proton labelled as H2 there are missing points in the area close to the diagonal due to the blind spot centred at proton Larmor frequency. The points were fit with linear regression. Dashed line ($|\nu_\alpha + \nu_\beta| = 2\nu_I$) represents the antidiagonal that crosses proton Larmor frequency point (14.71,14.71) in original HYSCORE map. The results of linear fit are summarized in Table S1.

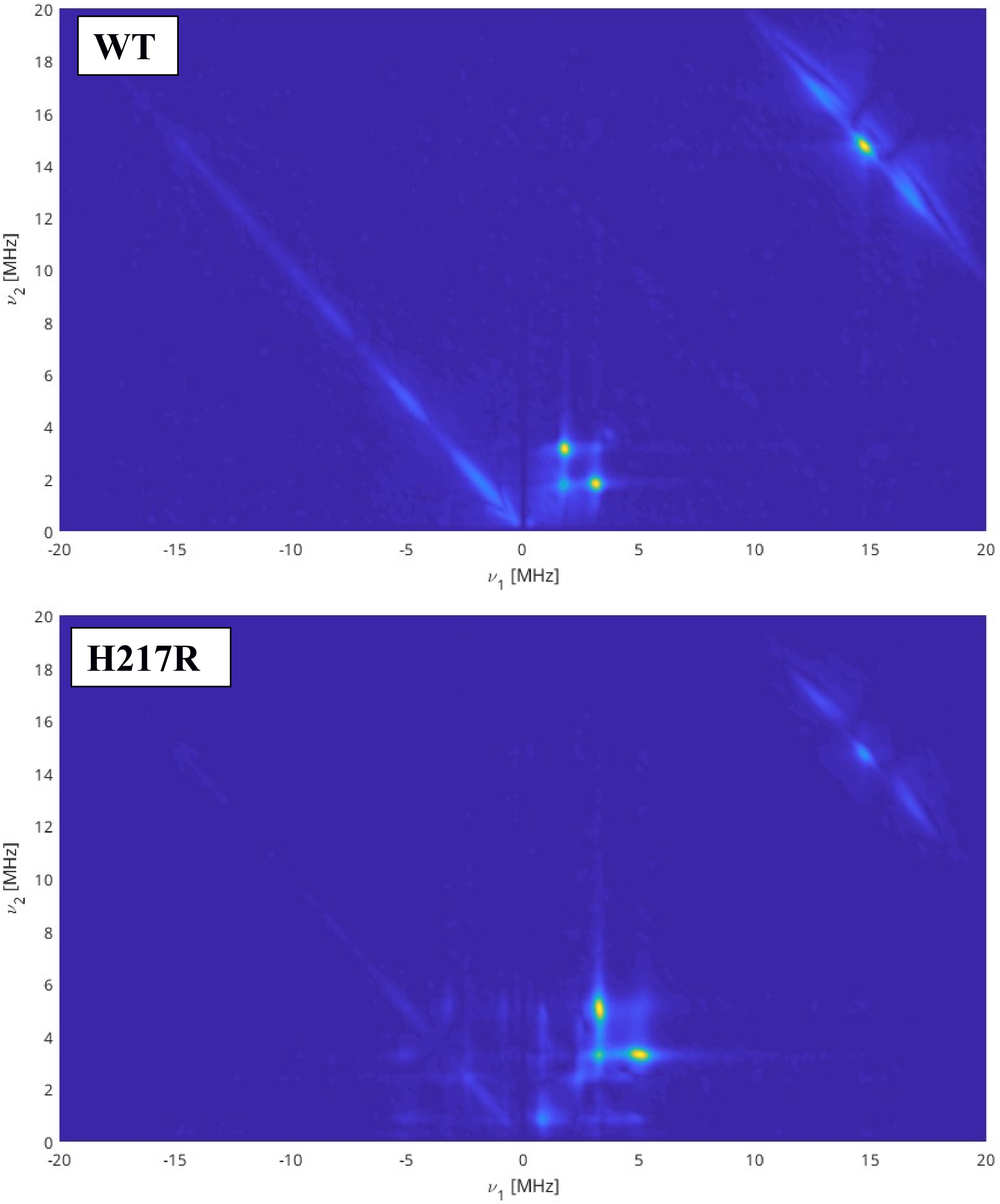

Fig. S4. X-band HYSCORE spectra of SQ$_i$ in WT (top) and H217R (bottom). Cross-peaks for ^{14}N (0 to 6 MHz) and ^{1}H (10 to 20 MHz) were present in (+,+) quadrant. No meaningful signals in (-,+) quadrant were present. The HYSCORE spectra were obtained at 345.5 mT (9.69 GHz) for τ = 136 ns. For detailed description of HYSCORE pulse sequence parameters please refer to Experimental section in main text.

Brief description of parameters obtained from HYSCORE analysis.

Hyperfine Interaction (HI)

The interaction between electron spin (S) and nuclear spin (I) is described in terms of spin hamiltonian by tensor A, which for typical axial symmetry is characterized by two parameters: a – the isotropic hyperfine coupling constant and T – dipolar coupling constant.
The principal values of tensor A (i.e. in its diagonal form) are related to a and T as follows:

$$A = \begin{pmatrix} A_1 & 0 & 0 \\ 0 & A_2 & 0 \\ 0 & 0 & A_3 \end{pmatrix} = \begin{pmatrix} a - T & 0 & 0 \\ 0 & a - T & 0 \\ 0 & 0 & a + 2T \end{pmatrix}$$

Isotropic part of the hyperfine interaction is referred to as Fermi contact interaction and is proportional to the unpaired spin density at the nucleus. The dipolar part (T) describes interaction between S and I through space and depends on distance between spins. More detailed description of HI parameters can be found for example in ref. [1].

Nuclear Quadrupole Interaction (NQI) is an interaction between electric quadrupole moment of the nucleus (only present when I > ½) and electric field gradient at the nucleus. NQI is characterized by traceless tensor Q:

$$Q = \begin{pmatrix} Q_1 & 0 & 0 \\ 0 & Q_2 & 0 \\ 0 & 0 & Q_3 \end{pmatrix} = \frac{e^2Qq/h}{4I(2I-1)} \begin{pmatrix} -1+\eta & 0 & 0 \\ 0 & -1-\eta & 0 \\ 0 & 0 & 2 \end{pmatrix}$$

For nucleus of I = 1 (^{14}N in our case) the constants in front the matrix evaluate to $\kappa = \frac{e^2Qq}{4h}$:

$$Q = \kappa \begin{pmatrix} -1+\eta & 0 & 0 \\ 0 & -1-\eta & 0 \\ 0 & 0 & 2 \end{pmatrix}$$

When a given spin system is in cancellation condition (A is twice the Larmor frequency of nuclei) the nuclear Zeeman interaction and hyperfine interaction cancel each other out in one electron spin manifold. In this case specific frequencies related only to NQI parameters appear in HYSCORE spectrum:

$$\nu_0 = 2\kappa\eta;\ \nu_- = \kappa(3-\eta);\ \nu_+ = \kappa(3+\eta)$$

These frequencies can be quickly identified as they follow simple addition rule: $\nu_0 + \nu_- = \nu_+$

Note: Please be aware that some authors define $\kappa = \frac{e^2Qq}{h}$ (i.e. the evaluated constants not divided by 4) and use another parameter $K = \frac{\kappa}{4}$ to express ν_0, ν_- and ν_+ frequencies. For more details on NQI see refs. [1,2].

Theory of HYSCORE proton region (squared-frequency coordinates) and fitting strategy

As shown by Dikanov [3], for axially symmetric hyperfine interaction (described by a, T), the squares of nuclear frequencies which are linked together in HYSCORE experiment follow the linear relation:

$$\nu_{\alpha(\beta)}^2 = \nu_{\beta(\alpha)}^2 Q_{\alpha(\beta)} + G_{\alpha(\beta)} \qquad (1)$$

where:

$$Q_{\alpha(\beta)} = \frac{2a + T \mp 4\nu_I}{2a + T \pm 4\nu_I} \qquad (2)$$

$$G_{\alpha(\beta)} = \pm \frac{4\nu_I^2 - a^2 + 2T^2 - aT}{2a + T \pm 4\nu_I} \qquad (3)$$

Because the HYSCORE map is symmetric, it actually does not matter to which axis we assign α i β frequencies. Therefore for clarity we can drop double indices for frequencies and rewrite the equation (1) into set of two equations:

$$\nu_\alpha^2 = \nu_\beta^2 Q_\alpha + G_\alpha \quad \text{(4a)}$$

$$\nu_\alpha^2 = \nu_\beta^2 Q_\beta + G_\beta \quad \text{(4b)}$$

which describe two ridges on the opposite side of the diagonal in the HYSCORE map. The signs of the *a*, *T* and ν_I in equations (2) and (3) determine whether the eqs. (4a) and (4b) represent the upper and lower ridge with respect to diagonal or vice versa. Also the relation between (4a) and (4b) is that:

$$Q_\alpha = \frac{1}{Q_\beta} \text{and} \quad G_\alpha = -\frac{G_\beta}{Q_\beta} \qquad (5)$$

Having these equations the fitting strategy was as follows. Two ridges of selected proton correlation pattern in HYSCORE map were fit independently giving parameters Q_α, G_α and Q_β and G_β. Then using eqs. (5) Q_β and G_β were recalculated into Q_α' and G_α' and the two parameters sets were averaged. Next in order to obtain *a* and *T* we used the equations from ref. [4]:

$$T = \pm \frac{4}{3(1 - Q_\alpha)} \sqrt{G_\alpha - G_\alpha Q_\alpha + 4\nu_I^2 Q_\alpha} \qquad (6)$$

$$a = 2\nu_I \frac{1 + Q_\alpha}{1 - Q_\alpha} - \frac{T}{2} \qquad (7)$$

which gave two sets of possible solutions. All results are summarized in Table S1.

Table S1. Parameters obtained from analysis of proton correlation patterns in HYSCORE map represented in squared-frequency coordinates. Parameters are defined in theory section.

	upper ridge		lower ridge		mean values[a]			
Proton	Q_α	G_α	Q_β	G_β	Q_α	G_α	T	(a,T)[b]
		MHz^2		MHz^2		MHz^2	MHz	MHz
H1	-1.246	483.9	-0.778	381.8	-1.266	487.3	±1.7	(-4.3,1.7) (-2.6,-1.7)
H2	-1.195	507.1	-0.852	428.9	-1.184	505.3	±5.4	(-5.2,5.4) (0.2,-5.4)

[a] the values of Q_β and G_β (lower ridge) were recalculated according to eqs. (5) and then averaged with the values obtained for upper ridge.

[b] as the absolute signs *a* and *T* cannot be determined in HYSCORE experiment, the other sets of solution are possible with opposite signs for each value.

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[2] S. Stoll, D. Goldfarb, EPR Interactions – Nuclear Quadrupole Couplings, in: EMagRes, John Wiley & Sons, Ltd, 2017: pp. 495–510. https://doi.org/10.1002/9780470034590.emrstm1504.

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[4] A.M. Weyers, R. Chatterjee, S. Milikisiyants, K.V. Lakshmi, Structure and Function of Quinones in Biological Solar Energy Transduction: A Differential Pulse Voltammetry, EPR, and Hyperfine Sublevel Correlation (HYSCORE) Spectroscopy Study of Model Benzoquinones, J. Phys. Chem. B. 113 (2009) 15409–15418. https://doi.org/10.1021/jp907379d.